Title: A pilot study assessing the implementation of 96 well plate-based aggregometry (Optimul) in Australia

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Running title
Optimul: platelet function testing in regional Australia

Abstract

Identification of disordered platelet function is important to guide peri-operative bleeding management as well as long term treatment and prognostic strategies in individual with platelet bleeding disorders. Light transmission aggregometry (LTA), the current gold standard diagnostic test of platelet function is a time-consuming technique almost exclusively performed in specialised laboratories and almost universally unavailable in regional centres in Australia, where there is an unmet need for access to specialised platelet function diagnostic services.

96-well plate based aggregometry, Optimul, has been utilised in research laboratories as a novel platform to investigate platelet function. We evaluated the Optimul assay at two centres in Australia, 1 regional and 1 tertiary metropolitan, to assess its feasibility as a screening test applicable to remote regional centres.

Concentration-response curves were established from 45 healthy volunteers at the participating regional hospital and from 31 healthy volunteers at the tertiary institution. Optimul successfully detected anti-platelet effects in individuals taking aspirin (n=4), NSAID (n=2), clopidogrel (n=2) and dual therapy with aspirin and clopidogrel (n=1). When tested in parallel to LTA in individuals referred for the evaluation of abnormal bleeding symptoms there was overall a very good level of agreement between Optimul and LTA (Cohen's kappa (k_2) =0.84) supporting its role as a useful screening tool in the assessment of platelet function. Optimul assay performance was quick and the methodology simple, requiring no specialised training or resources to be implemented at either the regional or metropolitan laboratory. Widespread implementation, particularly in regional laboratories within Australia where specialised platelet function testing is unavailable, has the potential to drastically improve the inequity of access to such services.

Keywords:

Optimul, platelet function testing, platelet bleeding disorders

1. Introduction

Inherited disorders of platelet function (IPFD) and/or number (IPND) are a heterogeneous group of conditions characterized by mucocutaneous bleeding of varying severity. Symptoms can be spontaneous or manifest in response to a haemostatic challenge such as childbirth, surgery, trauma and menstruation. Accurate and timely diagnosis is important for providing prognostic information to patients and families, to guide appropriate peri-operative bleeding management and to avoid the use of medications that increase bleeding risk.

Light transmission aggregometry (LTA), a technique developed in 1962 by Born [1] is considered the current "gold standard" for the *in vitro* testing of platelet function[2,3]. LTA measures the transmission of light through a sample of platelets in suspension, termed platelet rich plasma (PRP) and is based on the principal that light passes more easily through a clear solution when compared to a turbid solution. As platelet aggregates form in response to specific agonists, the turbidity of a solution is reduced, and light transmission is increased.

Traditional LTA has several advantages. It is well established, is relatively inexpensive and can demonstrate the kinetics of platelet responses including the two phases of platelet aggregation and platelet disaggregation in real time[4]. Despite these advantages, LTA has several logistical issues. LTA is time-consuming and requires expertise both in its performance and interpretation of aggregation traces. Although widely used, the LTA technique is not standardised across laboratories despite published guidelines aimed at improving standardisation[5,6]. Pre-analytical, methodological and interpretation variables all influence platelet aggregation results. Another major drawback is the large volume of blood required for LTA, which is particularly problematic in the paediatric population.

In 2011, Chan et al.,[7] designed and reported a new method of platelet reactivity testing using optical multichannel (Optimul) platelet aggregometry. The Optimul method uses a 96-well plate containing lyophilized agonists, each column of the plate contains a single agonist with decreasing concentration in each well down the column to which a small volume of patient PRP is added. Platelet activation is achieved by placement of the plate on a small bench-top high-speed thermoshaker that mixes PRP with agonist. Absorbance is then measured and the changes in absorbance are converted to percent aggregation with reference to the absorbance of PRP and PPP as 0% and 100% aggregation controls, respectively. Concentration-response curves for each agonist are subsequently constructed using fixed graphing protocols.

In 2014, Lordkipanidzé et al. compared the utility of Optimul in detecting platelet defects with LTA[8]. The authors found that the Optimul assay was 1) reproducible; 2) it could detect the effect of anti-platelet agents in healthy volunteers; 3) it could detect platelet defects in patients suspected of having a bleeding disorder; and 4) offered high sensitivity and high negative predictive value when compared to LTA. Importantly, advantages that include standardisation of agonists and agonist concentrations, incubation times, mixing technique, reading times, data acquisition and analysis, contribute to ease of assay performance. Furthermore, its ease and speed of use, small blood volume requirement (less than 2.5mL of PRP is required to produce full concentration-response curves to 7 agonists), its requirement for inexpensive equipment that is standardised across different laboratories lends itself as a cost-effective alternative for high throughput platelet function testing. It is particularly appealing in regional or rural settings, where specialised equipment is not available and could serve as a screening triage tool for referral to a tertiary centre for more specialised platelet function testing. We sought to determine whether the Optimul could be implemented successfully in a regional centre in Australia where access to specialised platelet function testing is currently not available.

2. Materials and Methods

All research was approved by the Northern NSW Local Health District Ethics Committee (2019/ETH00526) and the Northern New South Wales Sydney Human Research Ethics Committee (HREC/16/HAWKE/28). All participants gave written informed consent for participation in accordance with the Declaration of Helsinki.

2.1 Participating centres

Optimul was performed at two centres in NSW, Australia.
The first, Lismore Base Hospital (LBH) is a regional referral centre within the Northern New South Wales Local Health District (NNSW LHD), Australia. The LHD provides health care services to over 300,000 residents across a large area in north-eastern NSW. In 2011, the estimated population within the NNSWLHD was 288, 241, a number that is rapidly increasing. This centre has specialist haematology services offering patients a broad range of contemporary haemato-pathology laboratory tests, excluding specialised platelet function testing such as LTA.

The second centre, The Prince of Wales Hospital (POWH) is a large metropolitan tertiary referral centre providing specialist medical services to south-eastern Sydney and affiliated rural communities. Comprehensive on-site pathology services at the POWH includes ability to provide extensive platelet function testing.

2.2 Participants

2.2.1 Healthy volunteers

Healthy volunteers included those aged 18 or above, with no current or prior history of bleeding symptoms and those who had not administered any medication, food or complementary medicines known to impair platelet function in the preceding 2 weeks.

2.2.2 Individuals on medications known to affect platelet function

To assess the ability of Optimul to detect anti-platelet drugs, individuals taking aspirin (cyclooxygenase; COX) inhibitor, clopidogrel ($P2Y_{12}$ antagonist) and non-steroidal anti-inflammatories (NSAIDS; COX inhibition) were recruited. Medication lists were reviewed to ensure that these individuals were not taking other anti-coagulants or medication known to impair platelet function in the preceding two weeks.

2.2.3 Individuals referred for investigation of abnormal mucocutaneous symptoms

Individuals referred to the haematology services at both centres were invited to participate if the aetiology of their bleeding symptoms was uncharacterised after routine coagulation tests (at minimum prothrombin time, activated thromboplastin time, clauss fibrinogen, von Willebrand studies) and the individual did not have a diagnosis attributable to other causes of disordered platelet function such as medication, liver disease or renal disease.

2.3 Definition of diagnoses

Patients referred for evaluation of abnormal bleeding symptoms were ascribed diagnoses based on available laboratory and clinical data as follows:

a) Possible Platelet function disorder. An abnormal ISTH-BAT (male ≥4 points; female ≥6 points), Optimul panel and/or LTA in the absence of other disorders. Individuals meeting these criteria but with a concomitant disorder (e.g von Willebrand disease) or where medication affect (other than those commonly known to disrupt platelet

function)[9] could not be excluded (e.g selective serotonin reuptake inhibitors) were also categorized into this group.

b) Haemophilia, other single-factor deficiency or von Willebrand disease. Diagnosed according to current definitions [10-12]
c) Undefined Bleeding Disorder (UBD). A patient with abnormal mucocutaneous bleeding symptoms (ISTH-BAT: male ≥4 points; female ≥6 points) not meeting criteria for a) or b) above.
d) Systemic disorder/ other. Patients referred for evaluation of bleeding symptoms but where the ISTH-BAT score fell within normal limits (male ≤ 4 points; female < 6 points) and did not meet criteria for a) or b) above.

2.4 Assessment of platelet function

Blood was collected from the antecubital fossa, by clean venipuncture using minimal tourniquet pressure. The volume of blood drawn was dependent on whether Optimul alone (LBH) or Optimul in addition to LTA (POWH) was performed.

At both centres a single 2ml potassiumethylenediaminetetra-acid (EDTA) tube was filled for automated full blood count and peripheral blood film analysis. An additional 4-5x 2.7mL 3.2% buffered sodium citrate tubes (approximately 10-15mL) were taken for 96 well plate based aggregometry at LBH. At POWH 3x 2.7ml 3.2% buffered sodium citrate tubes were collected for anlaysis by Optimul, this was in addition to ~20-25mls citrated blood required for LTA that was performed as previously described [13]. For both Optimul and LTA, anti-coagulated blood from buffered sodium citrate tubes were centrifuged for the preparation of platelet-rich-plasma (PRP) and platelet-poor-plasma (PPP) according to published guidelines [9].
LTA was only performed at POWH as this assay was not available at LBH.

2.4.1 Optimul assay – 96 well plate based aggregometry

Optimul 96-well plates were prepared centrally, as previously described [14], by collaborators at The Blizard Research Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London. In brief, plates were pre-coated with hydrogenated gelatin in phosphate-buffered saline (PBS) to block the surface activation of platelets before addition of agonist. The agonists used were, AA (0.03-1 mM; Sigma-Aldrich, Poole, UK), ADP (0.005-40 μM; Sigma- Aldrich, Poole, UK), epinephrine (0.0004-10 μM; Labmedics, Stockport, UK), collagen (0.01-40 μg/mL; Nycomed, Linz, Austria), TRAP-6 amide (SFLLRN;

0.03-40 μM, Bachem, St. Helens, UK), U46619 (0.005-40 μM; Labmedics, Stockport, UK), and ristocetin (0.14-4 mg/mL; Helena Biosciences, Tyne and Wear, UK). The agonists were lyophilized by placing the plates in a -80 °C freezer for one hour then freeze-dried overnight at -40 °C, before being vacuum-sealed, foil-packed and kept at room temperature. The plates were couriered from the UK to Australia for use within 12 weeks of manufacture [14]. 40 μL of PRP or PPP was added into the corresponding agonist-free control wells of the 96-well plate. PRP was then added to the agonist-coated wells using a multi-channel pipette. The plate was subsequently placed on a high-speed shaker (BioShake IQ, Q instruments, Germany) to mix at 1200 rpm for 5 minutes at 37 °C. Absorbance was then measured at 595 nm on a 96-well plate reader (Azure Ao absorbance microplate reader, Azure Biosystems, USA) within 10 minutes of mixing.

2.4.2 Light transmission aggregometry (LTA)

Standard LTA was performed at the POWH as previously described [13]. Responses to the following panel of agonists were recorded using the Helena AggRAM (Helena Biosciences) four channel Aggregometer: ADP (1.25, 2.5, 5, 10 μM) (Sigma Aldrich); Horm collagen (0.5, 1, 2, 4 μg/mL) (Takeda, Germany); AA (0.8, 1.6 mM) (Helena Biosciences); epinephrine (2.5, 10 μM) (Sigma-Aldrich) and ristocetin (0.5, 1.2 mg/mL) (Helena Biosciences).

2.5 Data analysis

Agonist concentration-response curves were generated, and the curves fitted using fixed statistical and graphing protocols (GraphPad Prism™) as previously described [7,14,15]. Cohen's kappa statistic was used to measure agreement between LTA and Optimul [16].

3. Results

Concentration-response curves of platelet aggregation in healthy volunteers

96 well plate-based aggregometry was performed in 31 healthy volunteers using Optimul at the POWH. Typical sigmoidal shaped concentration-response curves were generated demonstrating a concentration-response relationship to all 7 agonists. Similarly, Optimul was

successfully employed at LBH in 45 healthy volunteers and generated typical concentration-response curves (Figure 1.).

Detection of antiplatelet agent effects by Optimul

Four individuals taking 100mg aspirin daily, 2 individuals taking NSAIDS (both ibuprofen 300mg, ≥ 1 dose within 24hrs of testing), 2 individuals taking clopidogrel 75mg daily and a single patient taking both aspirin and clopidogrel were tested by Optimul.

Aspirin irreversibly inhibits both COX-1 and COX-2. Likewise, ibuprofen is a non-specific COX inhibitor causing reversible inhibition of both COX-1 and COX-2. Typically, when assessed by LTA, inhibitors of COX prevent platelet aggregation in response to AA. Decreased aggregation to other platelet agonists (weak agonists such as epinephrine, low concentrations of collagen, and ADP) may also be seen, consistent with the positive feedback role of TXA2. Inhibition of platelet function was detected by Optimul in all 4 patients taking aspirin and both patients with recent NSAID use (Figure 2A). Platelet aggregation responses were markedly reduced to AA and moderately reduced to epinephrine. Milder abnormalities were noted to collagen and ristocetin.
Clopidogrel is an oral thienopyridine that selectively and noncompetitively blocks the $P2Y_{12}$ platelet receptor. Following oral ingestion much of the dose undergoes esterase deactivation. The remaining prodrug undergoes metabolism into the active drug moiety by the hepatic CYP450 (CYP3A4) system. Using LTA, inhibition of platelet aggregation induced by ADP, thromboxane analogs, and low concentrations of collagen and thrombin may be seen. Platelet inhibition to multiple agonists was observed in both patients taking clopidogrel. Markedly reduced aggregation to AA, moderately reduced response to epinephrine and mildly reduced responses to TRAP and U46619 were observed. Interestingly, in both patients' responses to ADP were not significantly different from control traces (Figure 2B). Predictably, combined aspirin and clopidogrel resulted in marked inhibition to several agonists including AA, collagen and epinephrine. ADP was moderately reduced and mild reduction in response to TRAP-6 and U46619 were observed (Figure 2C).

Optimul in individuals referred for evaluation of mucocutaneous bleeding symptoms

Platelet function was assessed by Optimul in 9 individuals referred for evaluation of abnormal bleeding symptoms at LBH (Table 1). All Optimul panels were plotted against the control-response curves and was defined as abnormal if the concentration-response curve was below the lower limit of the 95% reference interval for more than one agonist [8]. A

possible platelet function disorder was diagnosed in 2 individuals, a coagulation factor deficiency was identified in 1 individual, 2 patients were categorised as having UBDs and 2 patients as systemic disorder/other (Table 1).

Platelet function was assessed simultaneously by LTA and Optimul at the POWH (Table 2). Of the 12 patients evaluated, 5 were identified with abnormal LTA and Optimul traces and were assigned a diagnosis of a possible platelet function disorder. Normal traces to both LTA and Optimul were observed in 6 individuals. Five of these patients did not have increased bleeding scores and were assigned a diagnosis of symptoms secondary to systemic disorder/ other, 1 of these 6 patients was assigned a diagnosis of UBD. Abnormal platelet aggregation was demonstrated by Optimul in 1 patient (Table 2, patient 6), this result was discordant with LTA which showed normal aggregation to all agonists and normal agglutination to ristocetin. Interestingly, LTA performed previously for this patient at another institution had demonstrated a mildly abnormal platelet response to a single agonist (collagen). Overall, very good level of agreement was calculated between Optimul and LTA (Cohen's kappa (k_2) =0.84)[16].

Discussion

Platelet function testing is almost universally unavailable at centres outside of large metropolitan areas. This limits the ability to diagnose these bleeding disorders and provide appropriate bleeding prophylaxis and treatment.

Optimul is a novel alternative. Data to date indicates that it is sensitive and can produce accurate and standardized results [4,7,14,17]. Our pilot study demonstrated successful application of this platform at a regional centre in Australia. Performance of the assay alongside standard LTA confirmed very good concordance further supporting its potential use as an important tool in the diagnosis of individuals with platelet bleeding disorders.

Evaluation of the Optimul assay included determining whether the participating centres were able to generate control curves for healthy volunteers, as well as curves that reflected characteristic changes in platelet function in individuals taking anti-platelet medications (aspirin and clopidogrel) or those with anti-platelet affects (NSAIDs). Finally, responses in individuals referred for evaluation of bleeding symptoms were assayed.

Reproducible concentration-response relationships were achieved for all 7 agonists in healthy controls at both centres and reference intervals were established using an easily accessible graphing and statistical platform, GraphPad Prism™, employing non-parametric and robust statistics. However, as with LTA, reference intervals established at one centre

cannot be assumed to be transferable to another centre and authors recommend that control curves and reference intervals for Optimul be established for each agonist and agonist concentration from at least 20 samples at each centre [8,18-20].

In keeping with previous studies, Optimul was successful in detecting anti-platelet effects of aspirin, NSAIDS and clopidogrel. Somewhat surprisingly, responses to ADP in both patients taking clopidogrel were preserved, however, antiplatelet effect of the drug was apparent by significantly abnormal responses to other agonists, AA and epinephrine. Variable platelet responses to clopidogrel are well documented and may be secondary to several factors, which include noncompliance (not suspected in participants included in this study), variable absorption, metabolism and receptor sensitivity, and enhanced baseline platelet reactivity. Further evaluation of this observation however will need to be investigated in larger patient cohorts.

Optimul showed very good concordance with the “gold standard” LTA in those patients referred for evaluation of a suspected platelet bleeding disorder. Importantly in all cases where abnormal responses to platelet agonists were detected by LTA indicative of a possible platelet function defect, a concordant result was obtained by Optimul indicating excellent sensitivity of the assay. Interpretation of platelet function differed in a single case between assays (Table 2, individual 6). In that case, mild abnormalities to two platelet agonists were detected by Optimul whilst normal responses were obtained by LTA, indicating a possible false-positive result. Agreement between Optimul and LTA was seen in all other cases where no abnormality was detected by LTA. Consistent with previous reports [4] qualitative differences between LTA and Optimul were observed in our cohort reinforcing the recommendation that the two assays should be used as complementary tests of platelet function but that results are not strictly interchangeable [4].

Concentration-response curves were interpreted by haematologists and scientists with expertise in platelet function analysis. An Optimul panel was considered abnormal if the concentration-response curve was below the lower limit of the 95% reference interval for >1 agonist [8]. In cases where only mild abnormalities across different agonist concentrations are present interpretation of the concentration-response curves may be difficult. Here, if not available at the centre where Optimul was performed, analysis of the curves could be uploaded and sent for evaluation off-site until local experience is accumulated. Inter-observer reliability of the Optimul assay has not been studied and if expanded to additional sites would be an important measure to establish.

Considering implementation of the Optimul assay into a regional laboratory, we found that the Optimul assay successfully met several pre-requisites in terms of sample collection, test performance, staffing requirements/expertise and laboratory space. Optimul, like LTA, is a method that measures platelet responses to various platelet agonists in PRP from a fresh blood sample. It is subject to similar potential pre-analytical sources of variation. Optimul therefore requires a similar standardised approach to blood collection and preparation of PRP/PPP as is recommended in societal and international guidelines for LTA [5,9]. Precisely 2.25mls PPP/PRP is needed for each 96 well plate (40µl/well). An estimated 2-3x 2.7ml 3.2% buffered sodium citrate tubes of blood is required from each patient. To consider the possibility of procedural error, pipetting error, other unforeseen technical errors and to prevent participant call back owing to inadequate sample collection, we collected more than what would be required for testing at LBH (4-5 buffered sodium citrate tubes). In all cases this provided an adequate volume of PRP/PPP. Only 3 citrated blood tubes were collected for Optimul at POWH. This too provided sufficient plasma in all cases. We are confident that smaller volumes of blood (2-3 buffered sodium citrate tubes, equivalent to ~6mls whole blood) is sufficient volume for testing supporting the use of this platform in cases, such as in paediatric groups, where smaller blood volumes are desirable. Excluding the time required to prepare PRP (10mins) and PPP (15mins), test performance (pipetting, shaking and absorbance measurement) is less than 10 minutes per sample. In our experience, batch testing was possible. Four patient samples were easily collected and analysed during a typical testing session of approximately 2.5 hours. This was performed by a single staff member. Excluding concentration-response curve analysis, performance of the assay does not require specialised skills or operator expertise in platelet function testing. In our experience, the method was easily followed and competently performed by all laboratory staff after a single tutorial followed by observation of one assay then observed performance of a second assay. A very small amount of bench space is required for test performance. Excluding space required for PRP and PPP preparation and an absorbance reader, the BioShake IQ occupies a space of approximately 30cm by 40cm. Regarding information system support, Excel spreadsheet and GraphPad Prism™ are the only two programs required to generate concentration-response curves, the use of which required very minimal training. Unlike LTA where agonists must be used immediately once prepared, Optimul plates containing lyophilized agonist coated wells can be stored at room temperature, wrapped in aluminium foil for 12 weeks before use. This may suit smaller laboratories with unpredictable or low volume testing.

The financial cost of Optimul assay implementation should not be considered a barrier to widespread implementation, noting however that a detailed cost analysis has not been

undertaken. Each 96 well plate containing all 7 lyophilized agonists was purchased from our UK collaborators at The Blizard Research Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London for ~AUD 22.00. The only additional equipment required by both participating laboratories in this study was the BioShake IQ high-speed thermoshaker at a cost of ~AUD 2880.00, provided with a 2-year warranty. Other instruments (bench top laboratory centrifuge and absorbance reader) were already held by both laboratories. Additional costs included those standard reagents and consumables.

In summary, platelet function testing using Optimul 96 well plate based aggregometry was successfully piloted at two sites in Australia. Optimul has the potential to serve as a high throughput screening tool in regional and some metropolitan centres across the country. Additional work is planned to expand use of this platform to other regional sites across Australia as well as optimising standardised analytical, quality assessment and post-analytical procedures.

Acknowledgements

Gratitude is extended to Northern New South Wales Local Health district for supporting this work through the “Big ideas” innovation challenge grant (DR).

This work is also generously supported by the Thrombosis and Haemostasis Society of Australia and New Zealand (THANZ) Science and Education Research Grant (DR).

The views expressed in this manuscript are those of the authors and do not necessarily represent the views of the National Heart, Lung, and Blood Institute; the National Institutes of Health; or the US Department of Health and Human Services.

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Tables and figure legends

Table 1: Individuals referred for evaluation of platelet function

Patient	ISTH BAT score	Platelet count (x109/l)	VWF study results	Coagulation profile	Previous platelet function testing	Optimul result	Diagnosis
1	9	225	VWF:Ag, 118%; FVIII:C, 190%; VWF: RCo, 121%	PT 13s, aPTT35s, Fib 4.0g/l, FXI 124%, FXIII 113%	Nil	Normal aggregation to all agonists (confirmed by repeat testing)	UBD
2	2	325	VWF:Ag, 89%; FVIII:C, 97%; VWF: RCo, 76%	PT 12s, aPTT 24s, Fib 3.77g/l	Nil	Normal aggregation to all agonists	Systemic/ other
3	6	285	VWF:Ag, 78%; FVIII:C, 72%; VWF: RCo, 62%	PT 12s, aPTT 27s, Fib 3.39g/l, FXIII 151%	Nil	Mildly reduced response to ADP only	UBD
4	3	278	VWF:Ag, 99%; FVIII:C, 142%; VWF: RCo, 84%	PT 12s, aPTT 25s, Fib 3.07g/l, FXIII 141%	Nil	Normal aggregation to all agonists	Systemic/ other
5	8	281	VWF:Ag, 59%; FVIII:C, 64%; VWF: RCo, 63%; blood group O pos	PT 13s, aptt 34s, fib 2.5g/l, FIX 99%, FXI 70%, FXIII 111%	Nil	Normal (performed once)	UBD

6	19	201	VWF:Ag, 65%; FVIII:C, 69%; VWF: RCo, 62%; blood group: O pos	PT 13s, aPTT 30s, Fib 3.5g/l, FXI 104%, FXIII 158%	Reportedly abnormal - traces not seen	Mildly reduced responses to several platelet agonists - AA, ADP, epinephrine, TRAP-6, U46619.	Possible platelet function disorder. SSRI effect not excluded.
7	14	245	VWF:Ag, 43%; FVIII:C, 36%; VWF: RCo, 52%; blood group: ND	PT 13s, aPTT 35s, Fib 3.6g/l, FIX 99, FXI 85, FXII 118%%, FXIII 148%	PFA: Coll/ADP 147s (normal); Coll/Epi 210s (prolonged); Normal LTA: Collagen 2,5ug/ml; AA 0.5uM, ADP 10uM; normal ATP release by lumi-agg; mildly reduced dense granules (100 platelets, mean 2.33 (normal for lab 4-8), median 2, range 1-18, % normal 19%	Mildly reduced response to several agonists AA, ADP, U46619, ristocetin	Possible Platelet Function Disorder. Type I VWD.
8	4	210	VWF:Ag, 52%; FVIII:C, 66%; VWF: RCo, 51%; blood group O pos	PT 14s, aPTT 30s, Fib 2.7g/l, FXI 93%, FXIII 40% and 50% on repeat testing (lab range 58-181)	Nil	Normal	Possible mild FXIII deficiency
9	4	242	VWF:Ag, 111%; FVIII:C, 116%; VWF: RCo, 110%	PT 15s, aPTT 32s, Fib 2.5g/l, FIX 80%, FXI 96%, FXIII 107%%	Nil	Normal	UBD

PT =prothrombin time; aPTT=activated partial thromboplastin time, Fib= fibrinogen; VWF= von Willebrand factor ; RCo= ristocetin cofactor; SSRI= selective serotonin reuptake inhibitor; UBD= undiagnosed bleeding disorder.

Table 2: Patients assessed by LTA and Optimul

Patient	ISTH BAT score	LTA interpretation	Optimul interpretation	Interpretation of platelet function	Final diagnosis	Were LTA and Optimul concordant?
1	5	Reduced platelet aggregation to ADP at all concentrations and mildly reduced aggregation with epinephrine 10uM. Normal responses to other agonists. Normal agglutination to ristocetin	Mildly reduced aggregation to ADP, collagen, epinephrine and ristocetin	Signal transduction abnormality	Possible platelet function disorder	Yes
2	12	Reduced aggregation to collagen [0.5, 1.2 ug/mL], epinephrine, U44619 [2uM] and TRAP [15uM]	Reduced aggregation to collagen, epinephrine, TRAP and U44619	Dense granule deficiency (shown by WMEM)	Possible platelet function disorder	Yes
3	1	Normal aggregation to all agonists tested. Normal agglutination to ristocetin	Normal aggregation to all agonists. Normal agglutination to ristocetin	No definitive platelet function defect identified	Systemic/other	Yes
4	9	Normal aggregation to all agonists tested. Normal agglutination to ristocetin	Normal aggregation to all agonists. Normal agglutination to ristocetin	Dense granule secretion abnormality (supported by Lumi-Agg and WMEM)	Possible platelet function disorder	Yes
5	8	Normal aggregation to all agonists tested. Normal agglutination to ristocetin	Normal aggregation to all agonists. Normal agglutination to ristocetin	No definitive platelet function defect identified	UBD	Yes

6	2	Normal aggregation to all agonists tested. Normal agglutination to ristocetin. Previous testing - LTA - reduced response to low concentration collagen	Mildly reduced aggregation to collagen and mildly reduced with epinephrine and ADP	No definitive platelet function defect identified (inconclusive)	Systemic/other	No
7	not performed	LTA that showed - reduced aggregation to all concentrations of ADP, collagen and epinephrine. Normal to AA and ristocetin.	Abnormal responses to several agonists AA, ADP, collagen (mild), epinephrine, U46619, TRAP	Pathway undetermined (Lumi-Agg and WMEM not performed)	Possible platelet function disorder	Yes
8	5	Normal aggregation to all agonists tested. Normal agglutination to ristocetin	Normal aggregation to all agonists. Normal agglutination to ristocetin	No definitive platelet function defect identified	Systemic/other	Yes
9	5	Decreased responses to collagen and epinephrine with mild reduction to AA.	abnormal platelet responses to AA and Collagen	Dense granule deficiency (shown by WMEM)	Possible platelet function disorder	Yes
10	4	Normal aggregation to all agonists tested. Normal agglutination to ristocetin	Normal aggregation to all agonists. Normal agglutination to ristocetin	No definitive platelet function defect identified	Systemic/other	Yes
11	8	Reduced aggregation to Collagen 1ug/mL and epinephrine 10uM.	Mildly reduced responses to AA, collagen and U46619	Mild platelet dysfunction sec to SSRI	Possible platelet function disorder	Yes
13	3	Normal aggregation to all agonists tested. Normal agglutination to ristocetin	Normal aggregation to all agonists. Normal agglutination to ristocetin	No definitive platelet function defect identified	Systemic/other	Yes

LTA= light transmission aggregometry; Lumi-Agg= lumiaggreometry; SSRI= selective serotonin reuptake inhibitor; UBD= uncharacterised bleeding disorder; WMEM= whole mount electron microscopy.

Figure Legends

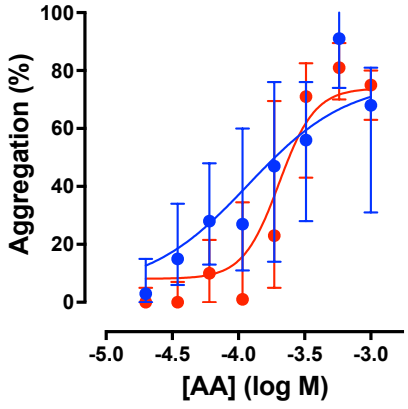
Figure 1: Concentration response curves established from healthy volunteers at both centres (LBH and POWH): Concentration-response curves in 45 healthy volunteers from LBH (red curve) and 31 healthy volunteers (POWH) (blue curve), presented as median and interquartile range. 95% confidence interval is shown by the broken lines.

Figure 2: Concentration response curves in patients taking anti-platelet agents. (A) Concentration-response curves obtained in 4 patients taking aspirin, presented as median and interquartile range (blue); and 2 patients taking non-steroidal anti-inflammatory agents, curves are shown separately for each of these individuals (red lines). (B) Concentration response curve of 2 patients taking clopidogrel (red curve). (C) Concentration response curve of 1 patient taking dual anti-platelet therapy (aspirin and clopidogrel). For curves (A), (B) and (C) concentration-response curves obtained from 45 healthy volunteers at LBH, presented as median and interquartile range (black). 95% confidence interval is shown by the broken lines (black)

Supplementary material

Video 1: An introduction to Optimul

Figure 1


Aggregation (%)
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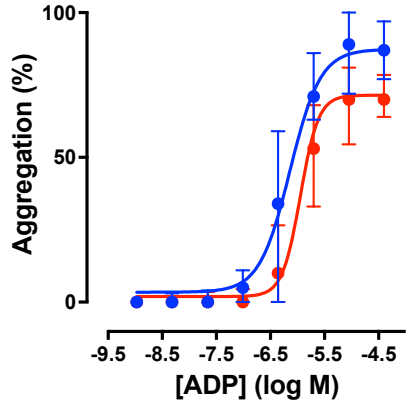
80
60
40
20
0
-5.0
-4.5
-4.0
-3.5
-3.0
[AA] (log M)


Aggregation (%)
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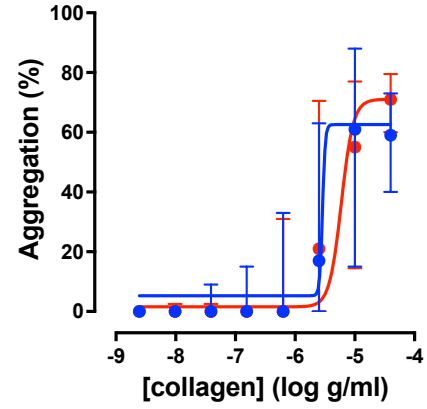
0
-9.5
-8.5
-7.5
-6.5
-5.5
-4.5
[ADP] (log M)


Aggregation (%)
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60
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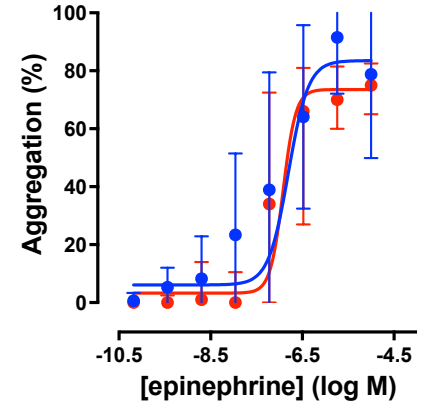
20
0
-9
-8
-7
-6
-5
-4
[collagen] (log g/ml)


Aggregation (%)
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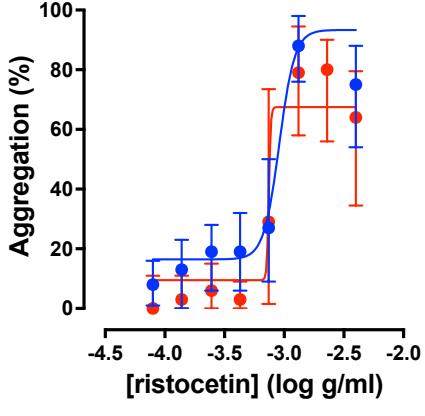
-8.5
-6.5
-4.5
[epinephrine] (log M)


Aggregation (%)
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80
60
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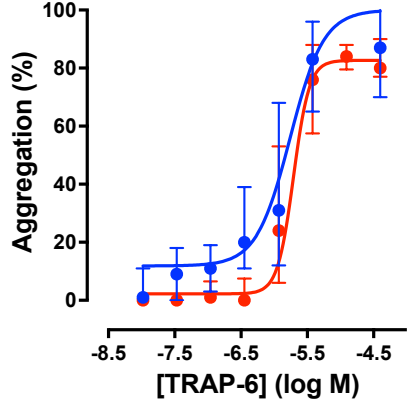
-4.5
-4.0
-3.5
-3.0
-2.5
-2.0
[ristocetin] (log g/ml)


Aggregation (%)
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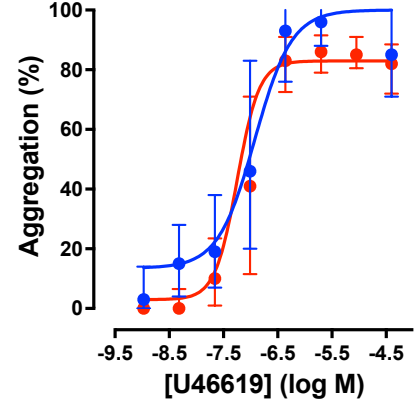
60
40
20
0
-8.5
-7.5
-6.5
-5.5
-4.5
[TRAP-6] (log M)


Aggregation (%)
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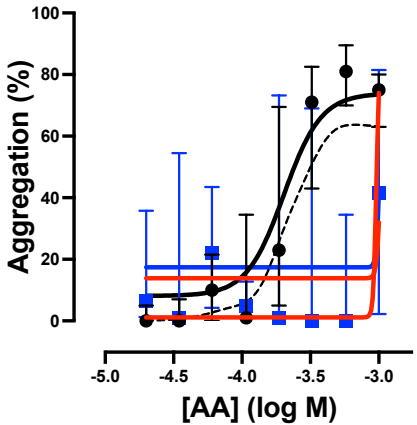
-5.5
-4.5
[U46619] (log M)

Figure 2 A


Aggregation (%)
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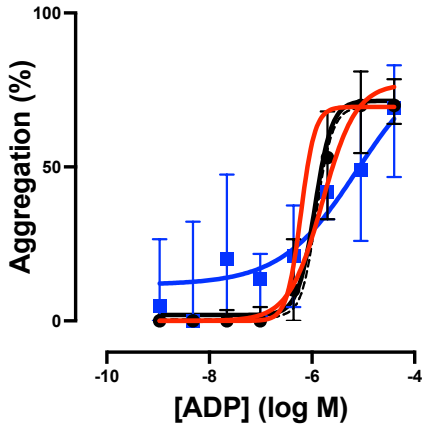
40
20
0
-5.0
-4.5
-4.0
-3.5
-3.0
[AA] (log M)


Aggregation (%)
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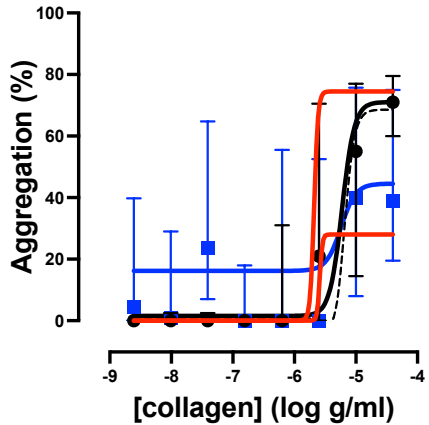
0
-10
-8
-6
-4
[ADP] (log M)


Aggregation (%)
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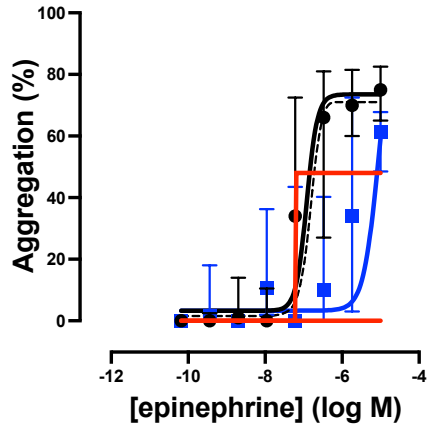
60
40
20
0
-9
-8
-7
-6
-5
-4
[collagen] (log g/ml)


Aggregation (%)
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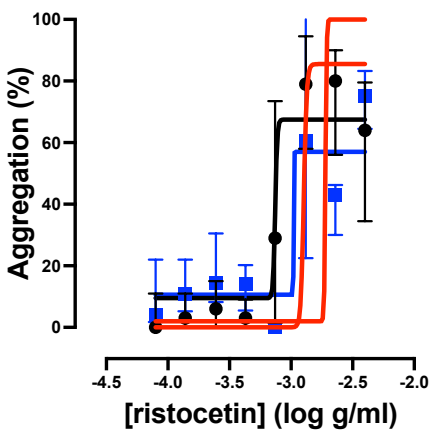
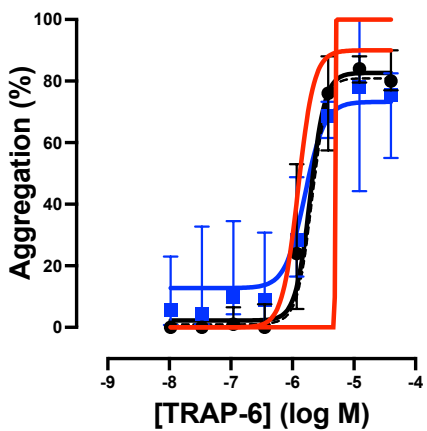
-12
-10
-8
-6
-4
[epinephrine] (log M)


Aggregation (%)
100
80
60
40
20
0
-4.5
-4.0
-3.5
-3.0
-2.5
-2.0
[ristocetin] (log g/ml)


Aggregation (%)
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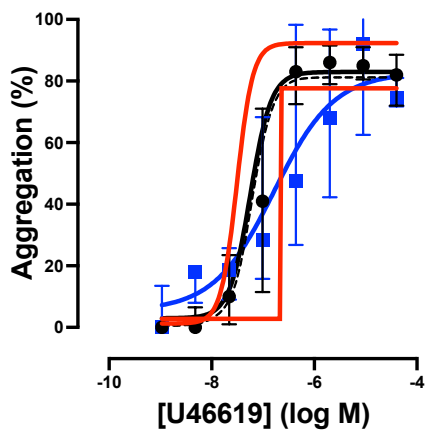
60
40
20
0
-9
-8
-7
-6
-5
-4
[TRAP-6] (log M)


Aggregation (%)
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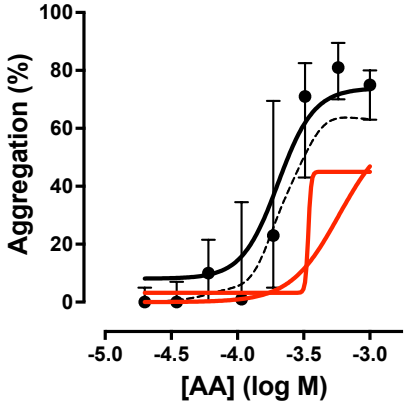
-10
-8
-6
-4
[U46619] (log M)

Figure 2 B


Aggregation (%)
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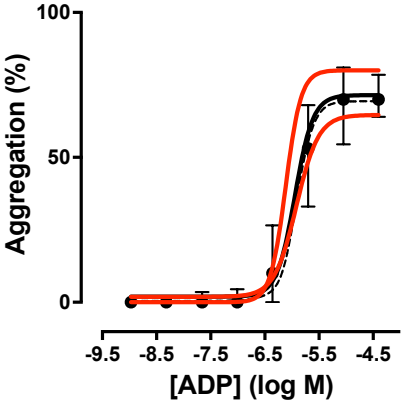
60
40
20
0
-5.0
-4.5
-4.0
-3.5
-3.0
[AA] (log M)


Aggregation (%)
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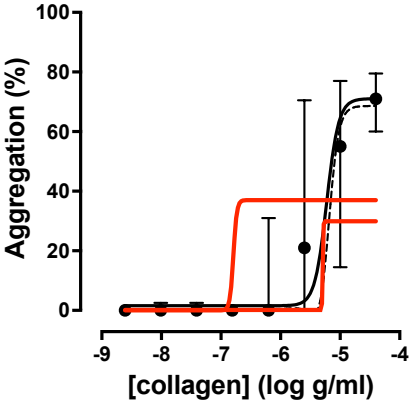
0
-9.5
-8.5
-7.5
-6.5
-5.5
-4.5
[ADP] (log M)


Aggregation (%)
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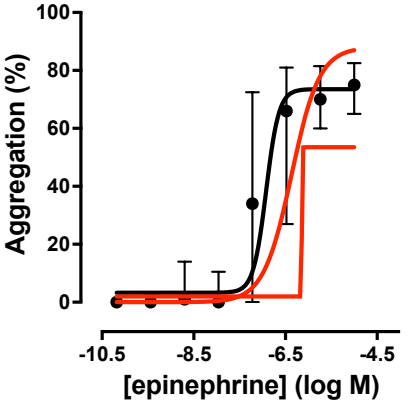
80
60
40
20
0
-9
-8
-7
-6
-5
-4
[collagen] (log g/ml)


Aggregation (%)
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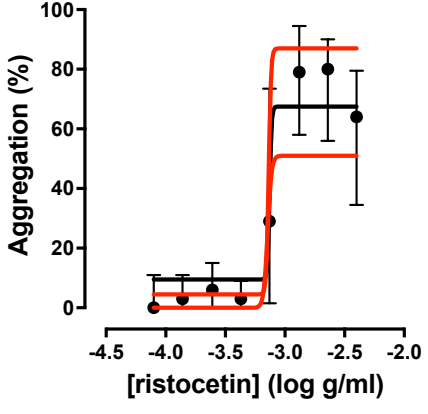
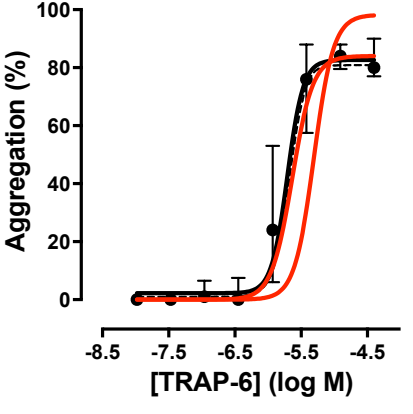
60
40
20
0
-10.5
-8.5
-6.5
-4.5
[epinephrine] (log M)


Aggregation (%)
100
80
60
40
20
0
-4.5
-4.0
-3.5
-3.0
-2.5
-2.0
[ristocetin] (log g/ml)


Aggregation (%)
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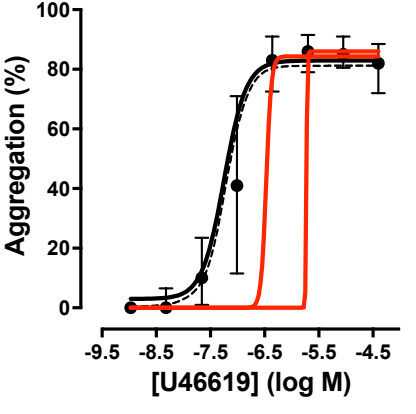
60
40
20
0
-8.5
-7.5
-6.5
-5.5
-4.5
[TRAP-6] (log M)


Aggregation (%)
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20
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-9.5
-8.5
-7.5
-6.5
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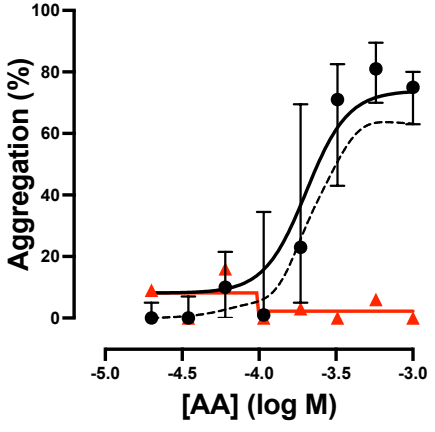
-4.5
[U46619] (log M)

Figure 2C


Aggregation (%)
100
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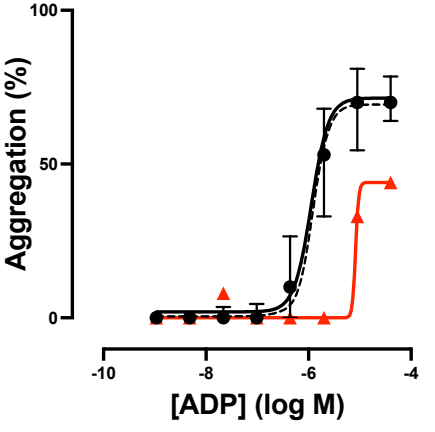
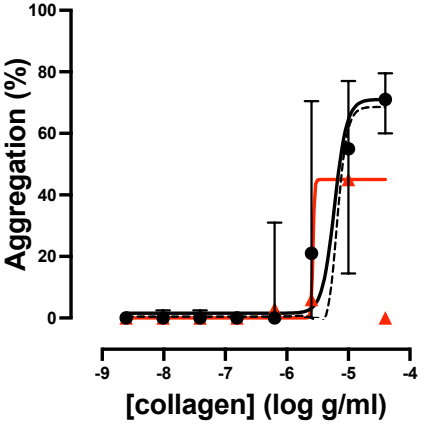
60
40
20
0
-5.0
-4.5
-4.0
-3.5
-3.0
[AA] (log M)


Aggregation (%)
100
50
0
-10
-8
-6
-4
[ADP] (log M)


Aggregation (%)
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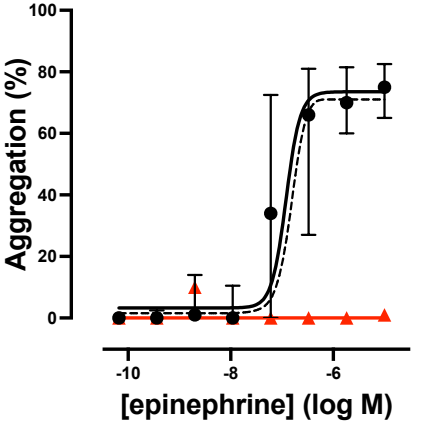
60
40
20
0
-9
-8
-7
-6
-5
-4
[collagen] (log g/ml)


Aggregation (%)
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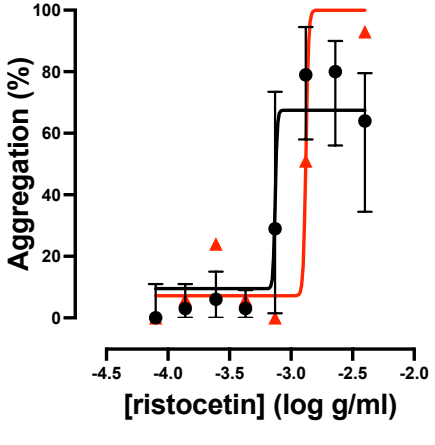
40
20
0
-10
-8
-6
[epinephrine] (log M)


Aggregation (%)
100
80
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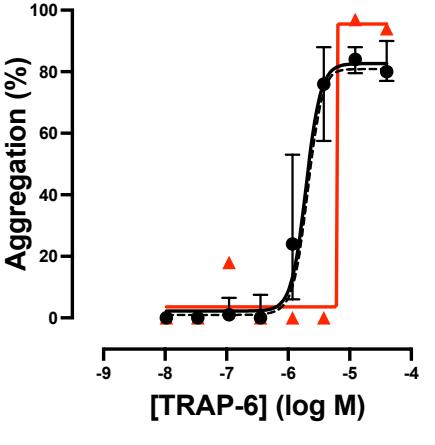
-4.5
-4.0
-3.5
-3.0
-2.5
-2.0
[ristocetin] (log g/ml)


Aggregation (%)
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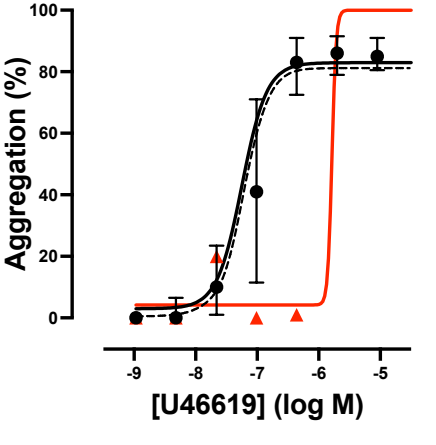
[TRAP-6] (log M)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-7
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[U46619] (log M)