OptiPrep™ Reference List RC02

Purification of platelets from whole blood/removal from blood leukocyte preparations

- OptiPrep™ is a sterile 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- This Reference List principally provides (in **Section 2**) a list of all papers reporting the use of OptiPrep™ in the purification of platelets from blood taken humans and from experimental animals and for their removal from leukocyte preparations. **Section 1** briefly summarizes the advantages of using the OptiPrep™ methodology.

1. The OptiPrep™ technology

The routine method for the purification of platelets from whole blood is to centrifuge the blood at a *g*-force that will sediment the erythrocytes and leukocytes, while leaving the smaller platelets suspended in the plasma supernatant. This is simple concept is technically very difficult; if the *g*-force is high enough to sediment all of the erythrocytes and leukocytes then many of the platelets will also be in the cell pellet; if it is sufficiently low to prevent the majority of platelets from pelleting then many of less dense leukocytes will also remain in the plasma supernatant. As a result the procedure needs to be repeated several times to recover more of the platelets from the pellet and to remove more leukocytes from the supernatant. It becomes a very tedious process.

In the one-step OptiPrep™ method, OptiPrep™ is diluted with a buffered saline to produce a solution of density 1.063 g/ml (lower than all of the leukocytes); an equal volume of whole blood is layered over it and centrifuged at 350 *g* for 15 min. The procedure is summarized in Figure 1. Typically 5 ml each of blood and the density barrier are used in a standard 15 ml tube. The separation is based on the much lower sedimentation rate of the platelets. The yields are approx. 90-92%. The method is equally effective for removing platelets from previously purified leukocyte fractions, particularly human peripheral blood mononuclear cells (PBMCs) produced by centrifugation of blood over a density barrier of approx. 1.077 g/ml (e.g. Lymphoprep™). A common method of removing the platelets is to dilute the cell harvest with saline and to use the lowest feasible *g*-force to pellet the cells selectively (usually about 300-350 *g* for 5-10 min). This is then repeated at least twice more – like the preparation of platelets by differential centrifugation (see above) this is inefficient, tedious and detrimental to the cells. Instead the platelets can be removed in a single step by layering the diluted harvest over the 1.063 g/ml barrier.

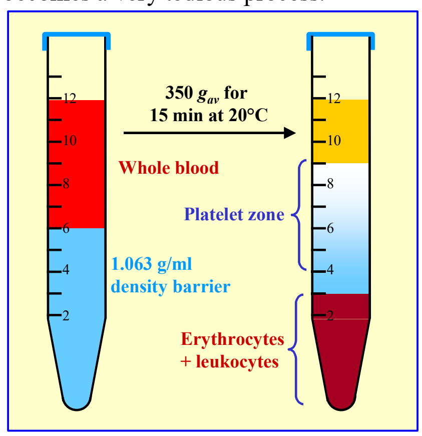

Figure 1: **Isolation of platelets from whole human blood**. (1) Equal volumes of blood and 1.063 g/ml density barrier layered in tube. (2) After centrifugation at 350 *g* for 15 min, platelets are harvested from the broad turbid band below the interface

The method is described in **OptiPrep™ Application Sheet (C13)**; this may be accessed from the Index of the "**Mammalian and non-mammalian cells**" file on the following website: www.OptiPrep.com (click on "Methodology").

2. Reference List

- The references are listed according to source of platelets and research topic. Within each section references are listed alphabetically by first author.

Canine platelets

Isolation and purification

Bonnefont-Rebeix, C., de Carvalho, C.M., Bernaud, J., Chabanne, L., Marchal, T. and Rigal, D. (2006) *CD86 molecule is a specific marker for canine monocyte-derived dendritic cells* Vet. Immunol. Immunopathol., 109, 167-176

Hennink, I., van Leeuwen, M.W., Penning, L.C. and Piek, C.J. (2018) *Increased number of tissue factor protein expressing thrombocytes in canine idiopathic immune mediated hemolytic anemia* Vet. Immunol. Immunopathol., **196**, 22–29
Trichler, S.A., Bulla, S.C., Thomason, J., Lunsford, K.V. and Bulla, C. (2013) *Ultra-pure platelet isolation from canine whole blood* BMC Vet. Res., **9**:144

Human platelets

Acetylcholinesterase expression

Camp, S., De Jaco, A., Zhang, L., Marquez, M., De La Torre, B. and Taylor, P. (2008) *Acetylcholinesterase expression in muscle is specifically controlled by a promoter-selective enhancesome in the first intron* J. Neurosci., **28**, 2459-2470

Activation

Bagamery, K. and Landau, R. (2005) *Different platelet activation levels in non-pregnant, normotensive pregnant, pregnancy-induced hypertensive and pre-eclamptic women. A pilot study of flow cytometric analysis* Eur. J. Obstet. Gynecol. Reprod. Biol., **121**, 117-123
Calzi, S.L., Purich, D., Chang, K., Afzal, A., Phillips, J., Agarwal, A., Segal, M. and Grant, M.B. (2007) *Cytoskeletal rearrangements via vasodilator-stimulated phosphoprotein (VASP) phosphorylation and redistribution in human retinal endothelial cells (HREC), endothelial precursor cells (EPC) and platelets: implications for retinal vessel repair* Invest. Ophthalmol. Vis. Sci., **48**, E-Abstr 4096
Natal, C., Restituto, P., Inigo, C., Colina, I., Diez, J. and Varo, N. (2008) *The proinflammatory mediator CD40 ligand is increased in the metabolic syndrome and modulated by adiponectin* J. Clin. Endocrinol. Metab., **93**, 2319-2327

α_2-Adrenoreceptors

Piletz, J., Baker, R. and Halaris, A. (2008) *Platelet imidazoline receptors as state markers of depressive symptomatology* J. Psychiatr. Res., **42**, 41-49

Aggregation

Onselaer, M-B., Eeckhoudt, S., de Meester, C., Hue, L., Vanoverschelde, J-L., Bertrand, L., Oury, C., Beauloye, C., Horman, S. (2010) *Calcium and calmodulin-dependent kinase kinase beta signalling controls human platelet aggregation* Circulation, **122**, Abstract 17868
Calzi, S.L., Purich, D., Chang, K., Afzal, A., Phillips, J., Agarwal, A., Segal, M. and Grant, M.B. (2007) *Cytoskeletal rearrangements via vasodilator-stimulated phosphoprotein (VASP) phosphorylation and redistribution in human retinal endothelial cells (HREC), endothelial precursor cells (EPC) and platelets: implications for retinal vessel repair* Invest. Ophthalmol. Vis. Sci., **48**, E-Abstr 4096
Calzi, S.L., Purich, D.L., Chang, K.H., Afzal, A., Nakagawa, T., Busik, J.V., Agarwal, A., Segal, M.S. and Grant, M.B. (2008) *Carbon monoxide and nitric oxide mediate cytoskeletal reorganization in microvascular cells via vasodilator-stimulated phosphoprotein phosphorylation evidence for blunted responsiveness in diabetes* Diabetes, **57**, 2488-2494

AMP-activated protein kinase

Onselaer, M-B., Oury, C., Hunter, R.W., Erckhoudt, S., Barile, N., Lecut, C., Morel, N. et al (2014) *The Ca^{2+}/calmodulin-dependent kinase kinase β-AMP-activated protein kinase-α1 pathway regulates phosphorylation of cytoskeletal targets in thrombin-stimulated human platelets* J. Thromb. Haemost., **12**, 973–986

Amyotrophic lateral sclerosis (sporadic)

Hishizawa, M., Yamashita, H., Akizuki, M., Urushitani, M. and Takahashi, R. (2019) *TDP-43 levels are higher in platelets from patients with sporadic amyotrophic lateral sclerosis than in healthy controls* Neurochem. Internat., **124**, 41–45

Antioxidative protection

Poniedzialek, B., Mleczek, M., Niedzielski, P., Siwulsk, M., Gasecka, M., Kozak, L., Komosa, A. and Rzymski, P. (2017) *Bio-enriched Pleurotus mushrooms for deficiency control and improved antioxidative protection of human platelets?* Eur. Food Res. Technol., **243**, 2187–2198

Atherogenesis

Heger, L.A., Hortmann, M., Albrecht, M., Colberg, C., Peter, K., Witsch, T., Stallmann, D., Zirlik, A., Bode, C., Duerschmied, D. and Ahrens, I. (2019) *Inflammation in acute coronary syndrome: Expression of TLR2 mRNA is increased in platelets of patients with ACS* PLoS One, **14**: e0224181

Bacterial infections/interactions

Arbesu, I., Bucsaiova, M., Fischer, M.B. and Mannhalter, C. (2016) *Platelet-borne complement proteins and their role in platelet–bacteria interactions* J. Thromb. Haemost. **14**, 2241–2252

Fejes, A.V., Best, M.G., van der Heijden, W.A., Vancura, A., Verschueren, H., de Mast, Q., Wurdinger, T. and Mannhalter, C. (2018) *Impact of Escherichia coli K12 and O18:K1 on human platelets: Differential effects on platelet activation, RNAs and proteins* Sci. Rep., **8**: 16145

Calumenin - thrombospondin-1 binding

Hansen, G.A.W., Vorum, H., Jacobsen, C. and Honore, B. (2009) *Calumenin but not reticulocalbin forms a Ca^{2+}-dependent complex with thrombospondin-1. A potential role in haemostasis and thrombosis* Mol. Cell Biochem., **320**, 25–33

Cancer cells, interactions with

Best, M.G., Sol, N., In 't Veld, S.G.J.G., Vancura, A., Muller, M., Niemeijer, A-L. N., Fejes, A.V., Fat, L-A.T.K., In 't Veld, A.E.H. Leurs, C. et al (2017) *Swarm intelligence-enhanced detection of non-small-cell lung cancer using tumor-educated platelets* Cancer Cell, **32**, 238–252

Bulla, S.C., Badial, P.R., Silva, R.C., Lunsford, K. and Bulla (2017) *Platelets inhibit migration of canine osteosarcoma cells* J. Comp. Path., **156**, 3-13

Da Costa Barros, T.A., de Oliveira Batista, D., de Carvalho, A.T., da Costa Faria, N.R., Barreto-Vieirae, D.F., Jácome, F.C., Barth, O.M., Ribeiro Nogueira, R.M. et al (2019) *Different aspects of platelet evaluation in dengue: Measurement of circulating mediators, ability to interact with the virus, the degree of activation and quantification of intraplatelet protein content* Virus Res., **260**, 163–172

Hayashi, Y., Jia, W., Kidoya, H., Muramatsu, F., Tsukada, Y. and Takakura, N. (2019) *Galectin-3 inhibits cancer metastasis by negatively regulating integrin β3 expression* Am. J. Pathol., **189**, 900-910

Hickish, T., Mohanty, P., Michael, S., Shivaswamy, S., Sunley, K., Varshney, A., Martin, R. and Simard, J. (2017) *Modulation of platelet levels by an anti-IL-1 α antibody (MABp1) in advanced colorectal cancer patients* Eur. J. Cancer, **72, Suppl.1**, S70

Zhang, X., Wang, J., Chen, Z., Hu, Q., Wang, C., Yan, J., Dotti, G., Huang, P, and Gu, Z. (2018) *Engineering PD-1-presenting platelets for cancer immunotherapy* Nano Lett., 5716−5725

Carbon monoxide

Calzi, S.L., Purich, D.L., Chang, K.H., Afzal, A., Nakagawa, T., Busik, J.V., Agarwal, A., Segal, M.S. and Grant, M.B. (2008) *Carbon monoxide and nitric oxide mediate cytoskeletal reorganization in microvascular cells via vasodilator-stimulated phosphoprotein phosphorylation evidence for blunted responsiveness in diabetes* Diabetes, **57**, 2488-2494

Coagulation factor

Dashty, M., Akbarkhanzadeh, V., Zeebregts, C.J., Spek, C.A., Sijbrands, E.J., Peppelenbosch, M.P. and Rezaee, F. (2012) *Characterization of coagulation factor synthesis in nine human primary cell types* Nat. Sci. Rep., **2**: 787

Community-acquired pneumonia

Laškaj, R., Salvica, D., Čepelak, I. and Kuzman, I. (2007) *Gamma-glutamyltransferase activity and total antioxidant status in serum and platelets of patients with community-acquired pneumonia* Arch. Med. Res., **38**, 424-431

Laškaj, R., Dodig, S., Čepelak, S. and Kuzman, I. (2009) *Superoxide dismutase, copper and zinc concentrations in platelet-rich plasma of pneumonia patients* Ann. Clin. Biochem., **46**, 123–128

Dengue virus haemorrhagic fever

Noisakran, S., Chokephaibulkit, K., Songprakhon, P., Onlamoon, N., Hsiao, H-M., Villinger, F., Ansari, A. and Perng, G.C. (2009) *A re-evaluation of the mechanisms leading to Dengue hemorrhagic fever* Ann. N.Y. Acad. Sci., **1171**, E24-E35

Tsai, J-J., Chang, J-S., Chang, K., Chen, P-C., Liu, L-T., Ho, T-C., Tan, S.S., Chien, Y-W., Lo, Y-C., Pern, G-C. (2017) *Transient monocytosis subjugates low platelet count in adult Dengue patients* Biomed Hub 2: 457785

Diabetic patients

Calzi, S.L., Purich, D.L., Chang, K.H., Afzal, A., Nakagawa, T., Busik, J.V., Agarwal, A., Segal, M.S. and Grant, M.B. (2008) *Carbon monoxide and nitric oxide mediate cytoskeletal reorganization in microvascular cells via vasodilator-stimulated phosphoprotein phosphorylation evidence for blunted responsiveness in diabetes* Diabetes, **57**, 2488-2494

Exosomes

Aatonen, M., Valkonen, S., Böing, A., Yuana, Y., Nieuwland, R. and Siljander, P. (2017) *Isolation of platelet-derived extracellular vesicles* In Methods Mol. Biol., **1545**, Exosomes and Microvesicles: Methods and Protocols (ed. Hill, A.F.) Springer Science+Business Media, LLC pp 177-188

Chiang, C-y. and Chen, C. (2018) *An increased level of CD41-positive extracellular vesicles recovered by 100,000 ×g centrifugation from stimulated platelets* J. Extracell. Ves., **7**, Suppl. 1, Abstr. # PF05.07

Factor VIII

Gilbert, G.E., Novakovic, V.A., Shi, J., Rasmussen, J. and Pipe, S.W. (2015) *Platelet binding sites for factor VIII in relation to fibrin and phosphatidylserine* Blood, **126**, 1237-1244

Meems, H., Meijer, A.B., Cullinan, D.B., Mertens, K. and Gilbert, G.E. (2009) *Factor VIII C1 domain residues Lys 2092 and Phe 2093 contribute to membrane binding and cofactor activity* Blood **114**, 3938-3946

Phillips, J.E., Lord, S.T. and Gilbert, G.E. (2004) *Fibrin stimulates platelets to increase factor VIIIa binding site expression* J. Thromb. Haemost., **2**, 1806-1815

Guanyl cyclase/cGMP signaling

Gambaryan, S., Kobsar, A., Hartmann, S., Birschmann, I., Kuhlencordt, P.J., Müller-Esterl, W., Lohmann, S.M. and Walter, U. (2008) *NO-synthase-/NO-independent regulation of human and murine platelet soluble guanylyl cyclase activity* J. Thromb. Haemost., **6**, 1376-1384

HPA-1a antigen

Ahlen, M.T., Husebekk, A., Killie, M.K., Skogen, B. and Stuge, T.B. (2009) *T-cell responses associated with neonatal alloimmune thrombocytopenia: isolation of HPA-1a–specific, HLA-DRB3*0101–restricted CD4^{+} T cells* Blood, **113,** 3838-3844

ILK-PINCH-Parvin complex signalling

Birschmann, I., Mietner, S., Dittrich, M., Pfrang, J., Dandekar, T. and Walter, U. (2008) *Use of functional highly purified human platelets for the identification of new proteins of the IPP signaling pathway* Thromb. Res., **122**, 59–68

Imidazoline receptors

Piletz, J., Baker, R. and Halaris, A. (2008) *Platelet imidazoline receptors as state markers of depressive symptomatology* J. Psychiatr. Res., **42**, 41-49

Metabolic syndrome

Natal, C., Restituto, P., Inigo, C., Colina, I., Diez, J. and Varo, N. (2008) *The proinflammatory mediator CD40 ligand is increased in the metabolic syndrome and modulated by adiponectin* J. Clin. Endocrinol. Metab., **93**, 2319-2327

Methodology

Bagamery, K., Kvell, K., Barnet, M., Landau, R. and Graham, J. (2005) *Are platelets activated after a rapid, one-step density gradient centrifugation? Evidence from flow cytometric analysis* Clin. Lab. Haem., **27**, 75-77

Bagamery, K., Kvell, K., Landau, R. and Graham, J. (2005) *Flow cytometric analysis of CD41-labeled platelets isolated by the rapid, one-step OptiPrep method from human blood* Cytometry Part A, **65A**, 84-87

Wrzyszcz, A., Urbaniak, J., Sapa, A. and Woźniak, M. (2017) *An efficient method for isolation of representative and contamination-free population of blood platelets for proteomic studies* Platelets, **28**, 43-53

Nitric oxide

Calzi, S.L., Purich, D.L., Chang, K.H., Afzal, A., Nakagawa, T., Busik, J.V., Agarwal, A., Segal, M.S. and Grant, M.B. (2008) *Carbon monoxide and nitric oxide mediate cytoskeletal reorganization in microvascular cells via vasodilator-stimulated phosphoprotein phosphorylation evidence for blunted responsiveness in diabetes* Diabetes, **57**, 2488-2494

Gambaryan, S., Kobsar, A., Hartmann, S., Birschmann, I., Kuhlencordt, P.J., Müller-Esterl, W., Lohmann, S.M. and Walter, U. (2008) *NO-synthase-/NO-independent regulation of human and murine platelet soluble guanylyl cyclase activity* J. Thromb. Haemost., **6**, 1376-1384

Prostanoid receptors

Coleman, R.A., Woodrooffe, A.J., Clark, K.L., Toris, C.B., Fan, S., Wang, J.W. and Woodward, D.F. (2019) *The affinity, intrinsic activity and selectivity of a structurally novel EP2 receptor agonist at human prostanoid receptors* Br. J. Pharmacol., **176**, 687–698

Proteomics

Rieckmann, J.C., Geiger, R., Hornburg, D., Wolf, T., Kveler, K., Jarrossay, D., Sallusto, F. et al (2017) *Social network architecture of human immune cells unveiled by quantitative proteomics* Nat. Immunol., **18**, 583-593
Tsai, J-J., Chang, J-S., Chang, K., Chen, P-C., Liu, L-T., Ho, T-C., Tan, S.S., Chien, Y-W., Lo, Y-C., Pern, G-C. (2017) *Transient monocytosis subjugates low platelet count in adult Dengue patients* Biomed Hub 2: 457785

Removal from cell preparations

Astley, S.B., Elliott, R.M., Archer, D.B. and Southon, S. (2004) *Evidence that dietary supplementation with carotenoids and carotenoid-rich foods modulates the DNA damage: repair balance in human lymphocytes* Br. J. Nutr., **91**, 63-72
Beppu, S., Nakajima, Y., Shibasaki, M., Kageyama, K., Mizobe, T., Shime, N. and Matsuda, N. (2009) *Phosphodiesterase 3 inhibition reduces platelet activation and monocyte tissue factor expression in knee arthroplasty patients* Anesthesiology; **111**,1227–1237
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Ivetic, N., Nazi, I., Karim, N., Clare, R., Smith, J.W., Moore, J.C., Hope, K.J., Kelton, J.G. and Arnold, D.M. (2016) *Producing megakaryocytes from a human peripheral blood source* Transfusion **56**, 1066–1074
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Pecher, G., Schirrmann, T., Kaiser, L. and Schenk, J.A. *Efficient cryopreservation of dendritic cells transfected with cDNA of a tumour antigen for clinical application* Biotechnol. Appl. Biochem., **34**, 161-166
Pecher, G., Häring, A., Kaiser, L. and Thiel, E. (2002) *Mucin gene (MUC1) transfected dendritic cells as vaccine: results of a phase I/II clinical trial* Cancer Immunol. Immunother., **51**, 669-673

Retinal vascular repair

Calzi, S.L., Purich, D., Chang, K., Afzal, A., Phillips, J., Agarwal, A., Segal, M. and Grant, M.B. (2007) *Cytoskeletal rearrangements via vasodilator-stimulated phosphoprotein (VASP) phosphorylation and redistribution in human retinal endothelial cells (HREC), endothelial precursor cells (EPC) and platelets: implications for retinal vessel repair* Invest. Ophthalmol. Vis. Sci., **48**, E-Abstr 4096

Serine residue, differential phosphorylation of

Calzi, S.L., Purich, D.L., Chang, K.H., Afzal, A., Nakagawa, T., Busik, J.V., Agarwal, A., Segal, M.S. and Grant, M.B. (2008) *Carbon monoxide and nitric oxide mediate cytoskeletal reorganization in microvascular cells via vasodilator-stimulated phosphoprotein phosphorylation evidence for blunted responsiveness in diabetes* Diabetes, **57**, 2488-2494

α-Synuclein

Barbour, R., Kling, K., Anderson, J.P., Banducci, K., Cole, T., Diep, L., Fox, M., Goldstein, J.M., Soriano, F., Seubert, P. and Chilcote, T.J. (2008) *Red blood cells are the major source of alpha-synuclein in blood* Neurodegener. Dis., **5**, 55-59

Thrombin stimulation

Hansen, G.A.W., Vorum, H., Jacobsen, C. and Honore, B. (2009) *Calumenin but not reticulocalbin forms a Ca^{2+}-dependent complex with thrombospondin-1. A potential role in haemostasis and thrombosis* Mol. Cell Biochem., **320**, 25–33

Thrombocytopenia

Ahlen, M.T., Husebekk, A., Killie, M.K., Skogen, B. and Stuge, T.B. (2009) *T-cell responses associated with neonatal alloimmune thrombocytopenia: isolation of HPA-1a–specific, HLA-DRB3*0101–restricted $CD4^{+}$ T cells* Blood, **113,** 3838-3844

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Tissue factor pathway

Girard, T.J., Grunz, K., Lasky, N.M., Malone, J.P. and Broze Jr, G.J. (2018) *Re-evaluation of mouse tissue factor pathway inhibitor and comparison of mouse and human tissue factor pathway inhibitor* physiology J. Thromb. Haemost., **16**, 2246–2257

Ventricular tachycardia

Josephs, K., Patel, K., Janson, C.M., Montagna, C. and McDonald, T.V. (2017) *Compound heterozygous CASQ2 mutations and long-term course of catecholaminergic polymorphic ventricular tachycardia* Mol. Genet. Genom. Med. **5**, 788–794

Primate bone marrow platelets

Dengue virus

Noisakran, S., Onlamoona, N., Hsiao, H-M., Clark, K.B,., Villingera, F., Ansaria, A.A. and Perng, G.C. (2012*) Infection of bone marrow cells by dengue virus in vivo* Exp. Hematol., **40**, 250–259

Rodent platelets

Aggregation

Camp, S., Zhang, L., Krejci, E., Dobbertin, A., Bernard, V., Girard, E., Duysen, E.G., Lockridge, O., De Jaco, A. and Taylor, P. (2010) *Contributions of selective knockout studies to understanding cholinesterase disposition and function* Chem-Biol. Interact., **187**, 72–77

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Platelet depletion

Hu, M., Li, S., Menon, S., Liu, B., Hu, M.S., Longaker, M.T. and Lorenza, H.P. (2016) *Expansion and hepatic differentiation of adult blood-derived $CD34^{+}$ progenitor cells and promotion of liver regeneration after acute injury* Stem Cells Translat. Med., **5**, 723-732

Platelet destruction *in vivo*

Bakchoul, T., Kubiak, S., Krautwurst, A., Roderfeld, M., Siebert, H.C., Bein, G., Sachs, U.J. and Santoso, S. (2011) *Low-avidity anti-HPA-1a alloantibodies are capable of antigen-positive platelet destruction in the NOD/SCID mouse model of alloimmune thrombocytopenia* Transfusion, **51**, 2455-2461

Platelet survival

Fuhrmann, J., Jouni, R., Alex, J., Zöllner, H., Wesche, J., Greinacher, A. and Bakchoul, T. (2016) *Assessment of human platelet survival in the NOD/SCID mouse model: technical considerations* Transfusion, **56**, 1370-1376

Thrombocytopenia model

Bakchoul, T., Fuhrmann, J., Chong, B.H., Bougie, D. and Aster, R. (2015) *Recommendations for the use of the non-obese diabetic/severe combined immunodeficiency mouse model in autoimmune and drug-induced thrombocytopenia* J, Thromb.Haemost., **13**, 1–4

Tissue factor pathway

Girard, T.J., Grunz, K., Lasky, N.M., Malone, J.P. and Broze Jr, G.J. (2018) *Re-evaluation of mouse tissue factor pathway inhibitor and comparison of mouse and human tissue factor pathway inhibitor* physiology J. Thromb. Haemost., **16**, 2246–2257

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