# Platelet activation test (PACT)

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## Abstract

Platelet activation was quantitatively assessed on a randomly selected subgroup of 10 patients (not treated with acetylsalicylic acid) in un-processed blood by the PACT (Platelet activation test, adjusted from Roest 2013). In the original paper, Roest performs the tests at room temperature while in this manuscript, the PACT test is performed at 37°C which requires higher agonist doses. The PACT is based on platelet activation induced by addition of a specific agonist to whole blood and gives specific insight in the granule release capacity and in the aggregation potential of platelets. The test contains three agonists to activate the platelets: (1) the protease activated receptor (PAR-1) agonist thrombin receptor activator peptide (TRAP f.c. 30 µM, SFLLRN, H-2936; Bachem, Germany), (2) the glycoprotein VI (GPVI) agonist collagen-related peptide (CRP f.c. 5 µg/ml, Pro- fessor Farndale, university of Cambridge, UK), and (3) the P2Y12 agonist ADP (f.c. 30 µM, 01897, Sigma-Aldrich, Zwijndrecht, the Netherlands) in HEPES-buffered saline (HBS, 10 mmol/L HEPES, 150 mmol/L NaCl, 1 mmol/L MgSO4, 5 mmol/L KCL, pH 7.4). The reaction mixtures also contain three antibodies directed against GPIb (APC-conjugated CD42b, BD Bioscience), activated αIIbβ3 (FITC-conjugated PAC-1) and P-selectin (PE-conjugated CD62P) purchased from BD Pharmingen (Franklin Lakes, USA). Whole blood was heated at 37°C for 10 min and the tests were performed at 37°C. Whole blood was diluted 1:4 in HEPES-buffered saline and 5 µl of this diluted blood was added to each reaction mixture. Reactions were stopped by adding 250 µl fixation solution (137 mmol/L NaCl, 2.7 mmol/L KCl, 1.12 mmol/L NaH2PO4, 1.15 mmol/L KH2PO4, 10.2 mmol/L Na2HPO4, 4 mmol/L EDTA, 0.5% formaldehyde) after exactly 20 min of incubation at 37°C. Flow cytometry was used to distinguish between platelets and other cells on forward and sideward scatter pattern and by gating on the CD42b positive cells. Fluorescent intensity in the FITC gate and PE gate was selected to determine activated αIIbβ3 and P-selectin density, respectively, and results are expressed as median fluorescent intensity (MFI).

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