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ELISA for measurement of platelet-activating factor (PAF) in serum.

Angel A Justiz-Vaillant¹, Belkis Ferrer-Cosme²

¹University of the West Indies St. Augustine;

²"Saturnino Lora Torres" Provincial Teaching Clinical Surgical Hospital.
Cuba

University of the West Indies

angel.vaillant@sta.uwi.edu

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Angel A Justiz-Vaillant

University of the West Indies St. Augustine

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Protocol status: In development
We are still developing and optimizing this protocol

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- 1 An anti-human PAF coating antibody is adsorbed onto the microwells by incubation overnight at 4°C with carbonate-bicarbonate buffer.

- 2 Add 50 µl of human serum or plasma. Human PAF present in the serum or plasma binds to antibodies adsorbed into the microwells.
- 3 The microplate is blocked with 3% non-fat milk-PBS buffer and later wash to remove unbound proteins.
- 4 Fifty (50) µl of biotin-conjugated anti-PAF antibody is added. The optimal dilution must be investigated.
- 5 The microplate is rewashed with PBS-Tween 20 buffer, pH 7.4.
- 6 One hundred µl of streptavidin-HRP conjugate is added and it binds to the biotin-conjugated anti-PAF antibody. The optimal dilution of this conjugate must be investigated.
- 7 The plate is washed following incubation to remove the unbound Streptavidin-HRP.
- 8 Add 100 µl of 3',3',5',5'- tetramethylbenzidine (TMB; Sigma-Aldrich) into each well.
- 9 Incubate the microwells in the dark for 20 min.

- 10** A colored product is formed in proportion to the quantity of PAF present in the sample or standard.
- 11** The reaction is terminated by addition of 100 μ l 3M H₂SO₄ and the absorbance is measured at 450 nm.
- 12** A standard curve is made from 7 human PAF standard dilutions and the human PAF sample concentration is determined.
- 13** For better results place the microplate on a microplate shaker in every incubation.