

**TEST: DETECTION OF THE PLATELET-SPECIFIC ANTIGEN-1a (HPA-1a) GENE POLYMORPHISM BY PCR**

**PRINCIPLE**

The human platelet alloantigens (HPA) system is important in the diagnosis of neonatal alloimmune thrombocytopenic purpura (NAITP). This is a severe neonatal disease caused by alloantibodies against fetal platelet antigens inherited from the father, which are not present in the mother, and which can cross the placenta and cause destruction of neonatal platelets. This can lead to cerebral bleeding in the neonate. There are five HPA systems, with HPA-1 being the most prevalent (75-85% of cases) followed by HPA-5 (10-20% of cases).

The HPA-1 system is a biallelic system (either a or b), characterized by the presence of thymidine or cytosine at base 196, respectively, which results in a leucine-to-proline substitution at position 33 of the protein.

The HPA-1a/b polymorphism can be identified by specific amplification of a 150-bp DNA fragment of *HPA-1* gene, by PCR. The amplification product is digested with *MspI* restriction enzyme. *MspI* does not digest the HPA-1a allele (the final product is 150-bp). *MspI* digests the HPA-1b allele into 95 and 55-bp products. The products can be visualized by gel electrophoresis.

**SPECIMEN COLLECTION AND PREPARATION:**

Collect 10ml blood by standard venipuncture techniques in **lavender top EDTA tubes**. Specimens should be delivered to the lab immediately or stored overnight at room temperature. Shipment to the laboratory should be by the same day or overnight, at room temperature. Peripheral blood specimens that are clotted, frozen or have not been collected in EDTA tubes are not acceptable.

**METHOD:** Polymerase chain reaction (PCR)

**REFERENCES:**

1. Forsberg et al. *Transfusion* 1995;35:241-46
2. Ouwehand G et al. *Arch Dis Fetal Neonatal Ed* 2000;82:F173-F175

**REPORTING RESULTS:**

The results are reported as follows:

<u>Results</u>	<u>Base Pairs</u>
HPA-1a/a	150
HPA-1a/b	150, 95, 55
HPA-1b/b	95, 55

**Turnaround time:** Two Weeks