TEST: **DETECTION OF THE FACTOR V H1299R (HR2) GENE POLYMORPHISM BY PCR**

PRINCIPLE

Coagulation Factor V is an enzyme cofactor that participates in the coagulation cascade and contributes to a normal haemostatic balance. Mutations in the *Factor V* gene are among the causes for venous thrombosis. Other conditions that are associated with mutations in the *Factor V* gene are pregnancy complications, such as recurrent pregnancy losses. The most common mutation in the *Factor V* gene is the Factor V Leiden mutation. Recently, another polymorphism in the *Factor V* gene, the His1299Arg polymorphism, has been identified and linked to hereditary thrombophilia.

The H1299R polymorphism can be identified by specific amplification of an 828-bp DNA fragment of *Factor V* gene, by PCR. The amplification product is digested with *Rsa*I restriction enzyme which yields two fragments of 447 and 381-bp for mutant alleles which can then 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. de Visser M et al. *Thromb Haemost* 2000;83:577-82
2. Castaman G et al. *Hematologica* 2003;88:1182-89

REPORTING RESULTS:

Digestion of the 828 bp amplified fragment from the wild type *Factor V* gene with *Rsa*I produces two fragments of 447 and 381 bp. The results are reported as follows:

Results	Base Pairs
Normal (1299HH)	828
Heterozygous (1299HR)	828, 447, 381
Homozygous (1298RR)	447, 381

Turnaround time: Two Weeks