Patient Name:	DUMMY	**Booking ID:**	NA
Age:	NA	**Sample Type:**	NA
Gender:	NA	**Sample Collection Date:**	DD-MM-YYYY
Referring Clinician:	NA	**Sample Receiving Date:**	DD-MM-YYYY
Test Requested:	Factor II Prothrombin gene mutation analysis by PCR, Sanger Sequencing	**Reporting Date:**	DD-MM-YYYY

FACTOR II PROTHROMBIN GENE MUTATION ANALYSIS

CLINICAL INDICATION

NA

RESULT SUMMARY

NEGATIVE
(Not Detected)

KEY FINDING

Target gene mutation	Mutation detection status	Relevance
Factor II - G20210A	Not Detected	None

RESULT INTERPRETATION

No mutation was detected for the Prothrombin Factor II gene variant G20210A.

Result classification	Comment
Homozygous mutation detected	Both copies of the gene carry mutation
Heterozygous mutation detected	One copy of the gene carries mutation
Not Detected	Mutation not detected

COMMENT

- ✓ Please correlate clinically.
- ✓ For about this report, or for assistance in locating nearby genetic counseling services, please contact the Laboratory: geneticcounselors@redcliffelabs.com, or ccsupport@redcliffelabs.com.

CLINICAL SIGNIFICANCE

Thrombosis is the formation of a blood clots inside a blood vessel, obstructing the blood flow of the cardiovascular system. Several thrombosis associated single nucleotide polymorphisms (SNPs) have been identified and reported to significantly increase the risk of deep venous thrombosis (DVT). Prothrombin (Factor II) gene (G20210A) has been found to be associated with increased Prothrombin levels and an increase in the risk for venous thrombosis in heterozygous. Higher concentrations of Prothrombin lead to increased rates of thrombin generation, resulting in excessive growth of fibrin clots. Heterozygosity for 20210G>A is associated with a 3-fold increased risk of venous thrombosis. Affected individuals may be candidates for anti-thrombotic prophylaxis. Homozygous are rare but two copies of the mutation would increase that risk. When heterozygosity for 20210G>A is combined with heterozygosity for the Factor V Leiden mutation, the relative risk for thrombosis increases further. Combination with non-genetic risk factors such as use of oral contraceptives, also leads to substantial elevations in relative risk.

TEST INFORMATION

This assay is based on DNA extracted from blood followed by PCR and targeted mutation Sanger Sequencing. The Test may be used for evaluation of patients with early onset VTE, as a thrombosis risk factor in patients prior to major surgery, to determine the cause of recurrent second or third trimester pregnancy loss, screening for risk of thrombosis before Oral contraceptive use and estrogen replacement therapy. Prothrombin G20210A mutation occurs in the noncoding region of the Factor II gene and is the second most common cause of inherited thrombophilia after FVL mutations. This test was developed and its analytical performance characteristics have been determined **by Redcliffe labs**. It has not been cleared or approved by FDA.

TEST LIMITATIONS

- ✓ Test results may vary if appropriate sample collection and transportation to lab not followed as per protocol.
- ✓ Mutations below the detection limits of the assay may not be detected. Typical detection limit for Sanger Sequencing assays is >10-20%.
- ✓ This test is laboratory developed and its performance were evaluated at National Reference Lab, Redcliffe Labs.
- ✓ PCR is a highly sensitive technique; reasons for apparently contradictory results may be due to improper quality control during sample collection, selection of inappropriate specimen and/or presence of PCR inhibitors.
- ✓ This test detects only ONE variants in Factor II gene and report includes variants that meets a level of evidence threshold for cause or contribute to disease.
- ✓ If this mutation is not found by the testing procedure, it does not mean that the risk of carrying or developing deep vein thrombosis is not present. It simply means that this specific mutation has not been found, although other mutations may be present.
- ✓ False positive or false negative results may occur for reasons that include genetic variants, blood transfusions, bone marrow transplantation, somatic or tissue-specific mosaicism.
- ✓ Gene transcript used for clinical reporting generally represents the canonical transcript, which is usually the longest coding transcript with strong/multiple supporting evidence.

DISCLAIMER

- Test has been performed assuming that the sample received belongs to the above-named individual(s) and that any stated relationships between individuals are accepted as true.
- The results should be interpreted in the context of the patient's medical evaluation. Mutation identified in this gene does not guarantee activity of the drug in a given indication due to presence of contraindicated mutation in gene.
- The mutation information provided should only be utilized as a guide or aid and the decision to select any therapy option based on the information reported here resides solely with the discretion of the treating physician.
- This report should only be used as an aid and the treating physician should employ sound clinical judgment in arriving at any decision for patient care or treatment.

REFERENCES

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2. Varga EA and Moll S. Prothrombin 20210 Mutation (Factor II Mutation). Circulation. (2004). 110:e15-e18.
3. Segers K, Dahlbäck B, Nicolaes GA. Coagulation factor V and thrombophilia: background and mechanisms. Thromb Haemost. (2007). 98(3):530-542.
4. Castoldi E, Simioni P, et al. Combinations of 4 mutations (FV R506Q, FV H1299R, FV Y1702C, PT 20210G/A) affecting the prothrombinase complex in a thrombophilic family. Blood. (2000). 96(4):1443-1448.

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Approved by
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Patient Name:	DUMMY	**Booking ID:**	NA
Age:	NA	**Sample Type:**	NA
Gender:	NA	**Sample Collection Date:**	DD-MM-YYYY
Referring Clinician:	NA	**Sample Receiving Date:**	DD-MM-YYYY
Test Requested:	Factor V Leiden Mutation Analysis by PCR,Sanger Sequencing	**Reporting Date:**	DD-MM-YYYY

FACTOR V LEIDEN MUTATION ANALYSIS

CLINICAL INFORMATION

NA

RESULT SUMMARY

NEGATIVE
(Not Detected)

KEY FINDINGS

Target gene mutation	Mutation detection status	Relevance
Factor V - H1299R	Not detected	None
Factor V -R506Q	Not detected	None
Factor V -Y1702C	Not detected	None

RESULT INTERPRETATION

No mutation were detected for Factor V gene variant H1299R, R506Q and Y1702C in given sample.

Result	Comment
Homozygous mutation detected	Both copies of the gene carry mutation
Heterozygous mutation detected	One copy of the gene carries mutation
Not Detected	Mutation not detected

COMMENT

- ✓ Please correlate clinically.
- ✓ For about this report, or for assistance in locating nearby genetic counseling services, please contact the Laboratory: geneticcounselors@redcliffelabs.com, or ccsupport@redcliffelabs.com.

CLINICAL INTERPRETATION

Thrombosis is the formation of a blood clots inside a blood vessel, obstructing the blood flow of the cardiovascular system. Several thrombosis associated single nucleotide polymorphisms (SNPs) have been identified and reported to significantly increase the risk of venous thrombosis. Three SNPs (R506Q, H1299R and Y1702C) in the Factor V gene are the most important genetic risk factors for inherited thrombophilia. Factor V mutation increases the relative risk of thrombosis by 5-10 fold in the heterozygous condition and by 50-100 fold in the homozygous individual. The lifetime risk for DVT is 12-20% for Heterozygote and 80% for Homozygote. Factor V mutation is a risk factor for venous as well as arterial thrombosis. It is the most common genetic risk factor for thrombosis and accounts for >90 percent of APC resistance.

TEST INFORMATION

This assay is based on DNA extracted from blood followed PCR and Sanger Sequencing. It is used as a thrombosis risk factor in patients prior to major surgery, to determine the cause of recurrent second or third trimester pregnancy loss, screening for risk of thrombosis before oral contraceptive use, estrogen replacement therapy and for presymptomatic evaluation of individuals with a family history of thrombosis or a family member identified to have FV mutations. A mutational defect in factor V causes APC (Activated Protein C) resistance which can be homozygous or heterozygous. Factor V Leiden mutation is a risk factor for venous as well as arterial thrombosis.

TEST LIMITATIONS

- ✓ Test results may vary if appropriate sample collection and transportation to lab not followed as per protocol.
- ✓ Mutations below the detection limits of the assay may not be detected. Typical detection limit for Sanger Sequencing assays is >10-20%.
- ✓ This test is laboratory developed and its performance were evaluated at National Reference Lab, Redcliffe Labs.
- ✓ PCR is a highly sensitive technique; reasons for apparently contradictory results may be due to improper quality control during sample collection, selection of inappropriate specimen and/or presence of PCR inhibitors.
- ✓ This test detects mutations only three target variants in Factor V gene and report includes only variants that meets a level of evidence threshold for cause or contribute to disease.
- ✓ Gene transcript used for clinical reporting generally represents the canonical transcript, which is usually the longest coding transcript with strong/multiple supporting evidence.

DISCLAIMER

- ❖ Test has been performed assuming that the sample received belongs to the above-named individual(s) and that any stated relationships between individuals are accepted as true.
- ❖ The results should be interpreted in the context of the patient's medical evaluation. Mutation identified in this gene does not guarantee activity of the drug in a given indication due to presence of contraindicated mutation in gene.
- ❖ The mutation information provided should only be utilized as a guide or aid and the decision to select any therapy option based on the information reported here resides solely with the discretion of the treating physician.
- ❖ This report should only be used as an aid and the treating physician should employ sound clinical judgment in arriving at any decision for patient care or treatment.

REFERENCES

1. Castoldi E, Lunghi B, et al. A missense mutation (Y1702C) in the coagulation factor V gene is a frequent cause of factor V deficiency in the Italian population. Haematologia. (2001). 86(6):629-633.
2. Ornstein DL and Cushman M. Factor V Leiden. Circulation. (2003). 107:e94-e97.
3. Ornstein DL, Cushman M, et al. The factor V HR2 haplotype and the risk of venous thrombosis: a meta-analysis. Journal of Hematology. (2003). 88(10):1182-1189.
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5. Castoldi E, Simioni P, et al. Combinations of 4 mutations (FV R506Q, FV H1299R, FV Y1702C, PT 20210G/A) affecting the prothrombinase complex in a thrombophilic family. Blood. (2000). 96(4):1443-1448.

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LABORATORY REPORT

Redcliffe labs

Patient Name:	DUMMY	**Booking ID:**	NA
Age:	NA	**Sample Type:**	NA
Gender:	NA	**Sample collection date:**	DD-MM-YYYY
Referring doctor:	NA	**Sample receiving date:**	DD-MM-YYYY
Test Requested:	MTHFR Variants (C677T, A1298C) mutation analysis by PCR, Sanger Sequencing method	**Reporting date:**	DD-MM-YYYY

MTHFR GENE MUTATION ANALYSIS

CLINICAL INDICATION

NA

RESULT SUMMARY

POSITIVE
(Detected)

KEY FINDING

Target gene mutation	Mutation detection status
MTHFR (NM_005957.5):c.1286A>C/ c.1298A>C	Homozygous mutation detected
MTHFR (NM_005957.5):c.665C>T/ c.677C>T	Not Detected

RESULT INTERPRETATION

Homozygous mutation was detected in MTHFR gene (NM_005957.5) for one variant i.e. c.1286A>C/c.1298 A>C.
No mutation was detected in MTHFR gene (NM_005957.5) for one variant i.e. c.665C>T/:c.677C>T.

Result zygosity classification	Comment
Homozygous mutation detected	Both copies of the gene carry mutation
Heterozygous mutation detected	One copy of the gene carries mutation
Not Detected	Mutation not detected

COMMENT

- ✓ Please correlate clinically.
- ✓ For about this report, or for assistance in locating nearby genetic counseling services, please contact the Laboratory: geneticcounselors@redcliffelabs.com, or ccsupport@redcliffelabs.com.

CLINICAL SIGNIFICANCE

Mutation in MTHFR (Methylenetetrahydrofolate reductase) is associated with hyperhomocysteinemia which is an independent risk factor for Stroke, Myocardial infarction, Peripheral arterial disease and venous thrombosis. Indian studies suggest that heterozygosity for MTHFR C 677T is also associated with elevated homocysteine levels. MTHFR C677T or A1298C carriers are not at increased risk for thrombosis in the absence of hyperhomocysteinemia. Homozygous MTHFR C 677T or A1298C carriers are at increased risk for hyperhomocysteinemia if they become deficient in vitamins B6, B12 or folic acid. Hyperhomocysteinemia is a relatively weak risk factor for both venous thromboembolism and arterial thrombosis.

COMMENT

A genetic polymorphisms commonly associated with severe MTHFR deficiency is defined by a C to T substitution (cytosine to thymine) at position 677 (C677T) of the MTHFR gene, which leads to the incorporation of amino acid alanine (A) instead of valine (V) at position 222 of the MTHFR protein. The altered MTHFR is known as “thermolabile MTHFR”. Homozygous and heterozygous carriers of this mutation both show reduced MTHFR activity. In particular, homozygous carriers suffer from significantly increased blood levels of homocysteine. C677T mutation in its homozygous form alone or as a compound heterozygote, which involves both C677T and an A1298C condition (where an Adenine (A) residue changes to a Cytosine (C) residue at the 1298th position) lead to the disruption of the MTHFR gene and causes a drastic reduction of the MTHFR enzyme. This in turn, leads to an elevation of Homocysteine in the blood. Homocysteine is an important substance in the blood as elevated levels of Homocysteine has been found to be the causative agent of various diseases such as; Cerebrovascular disease cerebral vein thrombosis, coronary artery disease, myocardial infarction, venous thrombosis neural tube defects leading to dementia and Alzheimer’s disease osteoporosis, diabetes, complications in pregnancy.

TEST INFORMATION

This assay is based on DNA extracted from peripheral blood or direct DNA followed by standard PCR and Sanger Sequencing of targeted variants. This test was developed and its analytical performance characteristics have been determined at Redcliffe labs. It has not been cleared or approved by FDA. Targeted sequencing and mutation analysis was performed by Polymerase Chain Reaction (PCR) followed by automated DNA sequencing of the amplicon using BigDye Terminator Chemistry on an ABI Genetic Analyzer 3500XL platform. Sequencing data were aligned to NCBI database to analyze the mutations.

TEST LIMITATION

- Test results may vary if appropriate sample collection and transportation to lab not followed as per protocol.
- Mutations below the detection limits of the assay may not be detected. Typical detection limit for Sanger Sequencing assays is >10-20%.
- This test is laboratory developed and its performance were evaluated at National Reference Lab, Redcliffe Labs.
- PCR is a highly sensitive technique; reasons for apparently contradictory results may be due to improper quality control during sample collection, selection of inappropriate specimen and/or presence of PCR inhibitors.
- This test detects only targeted gene variants and report includes variants that meets a level of evidence threshold for cause or contribute to disease.

- If this mutation is not found by the testing procedure, it does not mean that the risk of carrying or developing conditions is not present. It simply means that this specific mutation has not been found, although other mutations may be present.
- False positive or false negative results may occur for reasons that include genetic variants, blood transfusions, bone marrow transplantation, somatic or tissue-specific mosaicism.
- Gene transcript used for clinical reporting generally represents the canonical transcript, which is usually the longest coding transcript with strong/multiple supporting evidence.

DISCLAIMER

- Test has been performed assuming that the sample received belongs to the above-named individual(s) and that any stated relationships between individuals are accepted as true.
- The results should be interpreted in the context of the patient's medical evaluation. Mutation identified in this gene does not guarantee activity of the drug in a given indication due to presence of contraindicated mutation in gene.
- The mutation information provided should only be utilized as a guide or aid and the decision to select any therapy option based on the information reported here resides solely with the discretion of the treating physician.
- This report should only be used as an aid and the treating physician should employ sound clinical judgment in arriving at any decision for patient care or treatment.

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- Botto LD, Yang Q. 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: a HuGE review. Am J Epidemiol. 2000 May 1; 151 (9): 862 – 877.

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