The implementation of laboratory investigations for diagnosing pyruvate kinase deficiency at the Johannesburg Hospital

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DECLARATION

I declare that this report is my own work. It is being submitted for the degree of Master of Medicine (Clinical Pathology) at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other university.

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Pierre Durand

……. day of …….. 2007

DEDICATION

I dedicate this report to postgraduate students and researchers frustrated and discouraged by faculty administration

ABSTRACT

Pyruvate kinase is an essential enzyme in the anaerobic glycolytic pathway of the erythrocyte. The clinical presentation of this enzyme deficiency is due to the haemolytic process that results from the inability of erythrocytes to generate sufficient ATP. Although pyruvate kinase and glucose-6-phosphate dehydrogenase deficiencies comprise more than 90% of all reported red cell enzyme disorders worldwide, the epidemiology of the disease in South Africa is unknown and there is no assay for pyruvate kinase activity currently being used in South Africa. This report describes the implementation of screening and quantitative assays for pyruvate kinase activity in the Red Cell Membrane Unit at the University of the Witwatersrand Medical School / NHLS. The accuracy, precision and reproducibility of the assay were verified. Furthermore, a patient with pyruvate kinase deficiency was confirmed and found to have 15% of normal enzyme activity at 37^{o}C. The genetic abnormality was identified as a homozygous G1529A point mutation in exon 11 of the pyruvate kinase gene and to the candidate's knowledge is the first mutation described in a South African kindred. The patient's mother was heterozygous for the G1529A mutation and demonstrated an enzyme activity of 58% of normal at 37^{o}C.

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TABLE OF CONTENTS

CHAPTER 5: DISCUSSION

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

ACD – acid citrate dextrose

ADP – adenosine diphosphate

ARV – antiretroviral

ATP – adenosine triphosphate

AZT – azidothymidine

bp – base pair

CV – co-efficient of variation

DNA – deoxyribose nucleic acid

EA – enzyme activity

EDTA – ethylenediaminetetraacetic acid

G6PD – glucose-6-phosphate dehydrogenase

Hb – haemoglobin

HIV – human immunodeficiency virus

LDH – lactate dehydrogenase

NADH – nicotinamide adenine dinucleotide

NADPH - nicotinamide adenine dinucleotide phosphate

NHLS – National Health Laboratory Service

NRTI – nucleoside reverse transcriptase inhibitor

PBS – phosphate buffered saline

PCR – polymerase chain reaction

PEP – phosphoenolpyruvate

PK – pyruvate kinase

PK-LR – pyruvate kinase liver/red cell gene

SD – standard deviation

SOP – standard operating procedure

3TC – 2,3-dideoxy-3-thiacytidine

TE – Tris-EDTA