

# **DEVELOPMENT OF STRIP ASSAYS TO IDENTIFY COMMON BETA-THALASSAEMIA ALLELES IN MALAYS AND CHINESE IN MALAYSIA**

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## **INTRODUCTION**

Beta-thalassemia is the most common autosomal genetic disorder in Malaysia. Reduction or complete absence of  $\beta$ -globin chain synthesis is commonly due to mutations and small deletions in the  $\beta$ -globin gene. Varied clinical expressions ranging from severe life-threatening microcytic-hypochromic anaemia (thalassaemia major) to mild anaemia (thalassaemia trait) phenotypes are demonstrated depending on the level of beta globin chain production. The heterozygous carrier frequency of  $\beta$ -thalassaemia in Malaysia is estimated to be 4.5% from micro-mapping studies. It is a common public health problem in Malaysia, particularly among Malays and Chinese-Malaysians. The spectrum of  $\beta$ -thalassaemia mutations differs in each ethnic group in Malaysia. Four to five common mutations are responsible for more than 95% of the mutations seen in each ethnic group respectively. Knowledge of the common  $\beta$ -globin mutations in each ethnic group is crucial when designing rapid and cost-effective diagnostic tool for  $\beta$ -thalassaemia mutation detection. The current diagnostic method in Malaysia, amplification refractory mutation system (ARMS-PCR), is only able to identify one mutation in each reaction. It is found to be labour intensive and time consuming when few mutations need to be identified. Therefore, there is a need to have an effective and accurate laboratory method that can identify common mutations simultaneously in each ethnic group.

## **OBJECTIVES**

- 1) To develop a molecular tool to identify beta-thalassaemia mutations in Malays and Chinese-Malaysians
- 2) To develop reverse-dot blot hybridization (RDBH) strip assays to identify beta-thalassaemia alleles
- 3) To compare the RDBH strip assays with ARMS

## **METHODOLOGY**

In this study, the reverse dot blot hybridisation (RDBH) technique was used in development of strip assays for characterisation of the  $\beta$ -thalassaemia mutations. Two strip assays were designed specifically for Malays and Chinese-Malaysians respectively. Each strip is able to simultaneously identify six common mutations in each ethnic group. RDBH-Strip M(6) identified CD 26 (CAG  $\rightarrow$  AAG), IVS I-5 (G $\rightarrow$ C), IVS I-1 (G $\rightarrow$ T), CD 19 (A $\rightarrow$ G), CD8/9 (+G) and CAP+1 (A $\rightarrow$ C) in

Malays. RDBH-Strip C(6) identified CD41/42 (-TTCT), IVS II-654 (C→T), CD17 (A→T), -28 (A→G), -29 (A→G) and CD71/72 (+A) in Chinese-Malaysians. These selected panels of  $\beta$ -globin gene mutations were based on the mutation frequencies reported in previous studies in Malaysia. The mutations identified with the strip assays were validated with the gold standard method, ARMS-PCR.

## RESULTS

A total of 177 patients (354 alleles) from University Malaya Medical Centre (UMMC) and the Institute of Medical Research (IMR) in Malaysia were studied. One hundred and thirty seven were Malays (274 alleles) and 40 were Chinese-Malaysians (80 alleles) respectively. One hundred and nineteen (86.9%) Malay patients consisting of 238 alleles were identified by the RDBH-Strip M (6). In the Malays, the most common  $\beta$ -thalassaemia mutations identified was CD 26, followed by IVS I-5, IVS I-1, CD 19 and the least with CD 8/9. In view of possible inter-marriage with Chinese, the RDBH-Strip C (6) was used to identify the 18 unidentified alleles in the Malays. The mutations identified were common Chinese mutations, CD 41/42 (5 heterozygous), CD 17 (2 heterozygous), -29 (2 heterozygous) and CD 71/72 (1 homozygous). Thus, a total of 129 (94.6%) Malay patients consisting of 258 alleles were identified using the RDBH-Strip Assays [RDBH-Strip M (6) and RDBH-Strip C (6)]. In the Chinese-Malaysians by the RDBH-Strip C (6), mutations were identified in 32 (80%) patients consisting of 64 alleles. IVS II-654 and CD 41/42 were the two most common  $\beta$ -thalassaemia mutations amongst Chinese-Malaysians, followed by CD17 and -28. In the Chinese-Malaysians, RDBH-Strip M (6) identified CD 26 (3 heterozygous) and IVS I-5(1 heterozygous). Thus, a total of 36 (90.0%) Chinese-Malaysians patients consisting of 72 alleles were identified using the RDBH-Strip Assays [RDBH-Strip C (6) and RDBH-Strip M (6)]. ARMS-PCR was used to confirm and validate the presence of the six mutations used in RDBH-Strip Assays. It amplified each mutation as a separate and distinct PCR product. The Strip Assays showed 100% sensitivity and specificity through validation by ARMS-PCR. Therefore, the Strip Assays developed can be defined as a reliable diagnostic tool for accurate beta-thalassaemia mutation identification in Malays and Chinese Malaysians. Unidentified mutations in the eighteen Malays and eight Chinese-Malaysian patients by RDBH-Strip M(6) and RDBH-Strip C(6) were simultaneously tested with a commercialized  $\beta$ -globin strip assay SEA (Southeast-Asian type) (Viennalab diagnostics GmbH, Austria). In the Malays, the mutations identified were five patients heterozygous for CD 41/42, two heterozygous for -29 and CD 17 respectively and one homozygous for CD 71/72. These results were in concordance with the result tested using RDBH-Strip C (6). In the Chinese-Malaysians, the commercialised  $\beta$ -globin strip identified three patients heterozygous for CD26, one heterozygous for IVS1-5 and one with an initiation codon defect (ATG→AGG). The initiation codon defect is a rare mutation in Chinese-Malaysians. There were remaining 11 heterozygous beta-thalassaemia carriers (eight Malays and three Chinese) whose mutations could not be identified. These unknown mutations require DNA sequencing for ultimate diagnosis. The RDBH-Strip Assays developed in this project study identified 93.2% of the mutations seen in the Malays and Chinese-Malaysians.

## CONCLUSION

In conclusion, the developed RDBH- Strip Assays [M (6) and C (6)] are accurate and rapid diagnostic tools for the identification of beta-thalassaemia mutations in the Malays and Chinese-Malaysians.

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