Sickle cell disease is an important inherited blood disorder. Universal screening and early intervention have significantly helped to reduce childhood mortality in industrialized countries. However in low-resource settings children are often not diagnosed until late childhood, when clinical symptoms are presenting. Current diagnostic techniques are highly accurate and mostly based on isoelectric focusing, HPLC or mass spectrometry, which require advanced laboratory equipment. A simple and rapid molecular diagnostic test could be implemented in small laboratories and developing countries where such advanced equipment are not available.

INTRODUCTION

The Human Hemoglobin S/C kit uses loop-mediated isothermal amplification (LAMP) with melting curve analysis for rapid and accurate detection of hemoglobin S and C. The test is performed directly on fresh or frozen blood samples, or on dried blood spots. The kit contains a ready to use mastermix, to which samples are added after lysis. The complete protocol has only two manipulation steps, as presented in Figure 1 and results are obtained within 45 minutes.

In this study 50 fresh blood samples and 100 dried blood cards from routine diagnostics have been tested with the proposed LAMP method. Molecular results were compared with the corresponding phenotypic assays performed in the genetics lab at the university hospital of Liège. Capillary electrophoresis was used as reference method for whole blood samples while tandem mass spectrometry was preferred for dried blood cards.

MATERIAL AND METHODS

The Human Hemoglobin S/C kit uses loop-mediated isothermal amplification (LAMP) with melting curve analysis for rapid and accurate detection of hemoglobin S and C. The test is performed directly on fresh or frozen blood samples, or on dried blood spots. The kit contains a ready to use mastermix, to which samples are added after lysis. The complete protocol has only two manipulation steps, as presented in Figure 1 and results are obtained within 45 minutes.

RESULTS

Results of the Hemoglobin S/C kit are presented in a graph, with automatic interpretation of the melting temperatures. A run is only considered as valid as the positive and negative control are within the prescribed settings. The positive control is a plasmid control containing HbC, Hba and Hbs.

Twenty-six runs were performed in this study, each including six samples, a positive control and a negative control. One run showed incorrect results for the positive control and results were not accepted. The run was repeated and correct results were obtained.

One sample with genotype AS was falsely identified as AA (Blue line on Figure 2). The sample was repeated and the correct genotype was obtained. On the graph it was clear that the sample should be repeated, as a small S-peak was visible with the eye but was not detected by the software. Therefor it is important that interpretation of the result is done with the melting temperatures in combination with the graph image.

CONCLUSION

86% of the blood sample results were in complete correspondence with the result of the routine diagnostic method of the university hospital of Liège. The relative low number is due to the inclusion of different genotypes that cannot be identified with the molecular assay (Table 1). Genotype AD was identified as AA, βthal- and HPFH were identified as AA, Sβthal was identified as AS. For the DBS samples 98% of the results corresponded to the result of the routine analysis, only one sample with genotype AE was identified as AA.

In total 94% of the results were in complete agreement with the results of the routine analysis (Figure 3). When only the genotypes included in the Hemoglobin S/C assay are considered only 1/150 samples tested was genotyped incorrectly.

Genotypes HbSS, HbAS, HbAC, HbAA and HbSC can be correctly identified with the Human Hemoglobin S/C kit. Other hemoglobin variants like Hb D or Hb E and β-globin production defects (β-thalassemia) could not be identified with this technique, as expected. When using the HemoglobinS/C Kit as screening assay it is important that heterozygous samples are confirmed with another technique in which all genotypes are included.

The assay performs well on both fresh blood samples and dried blood spots, with limited input volumes. Based on the study results this technique is robust and accurate and can be used for sickle cell disease screening from blood and dried blood spots, with minimal hands-on time and minimal laboratory equipment. Especially for dried blood spots the limited manipulation steps are unique for molecular methods.