

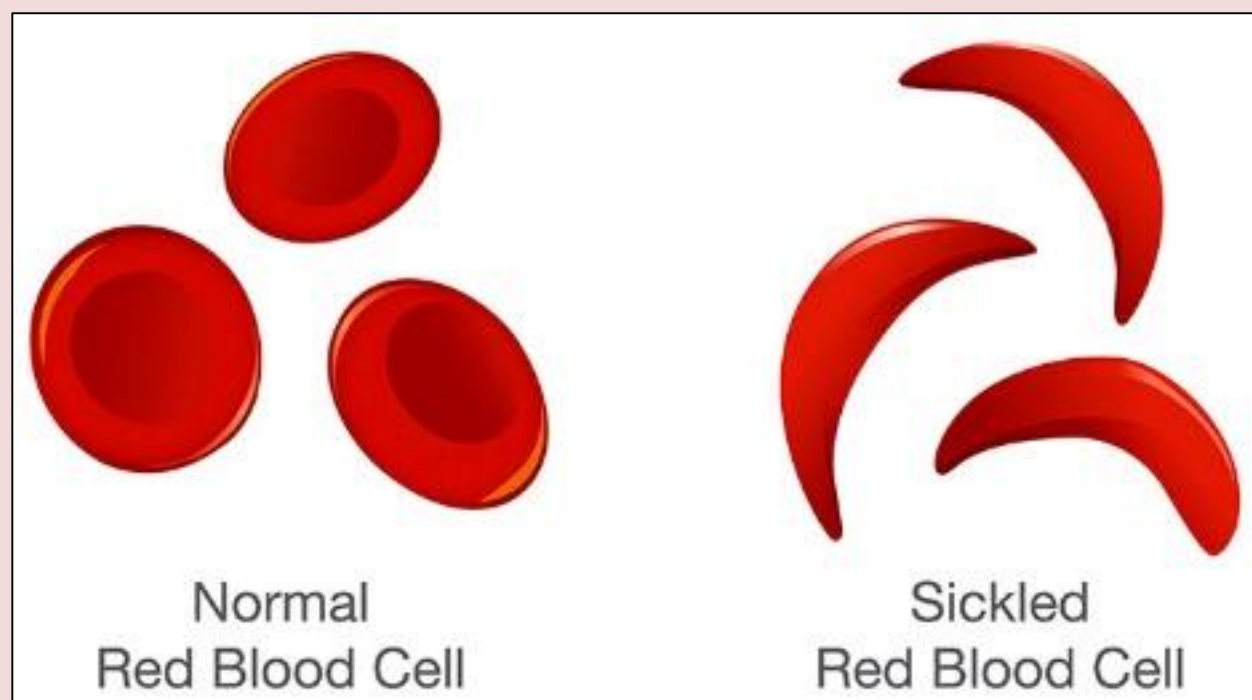


Appropriate blood provision for Sickle Cell Disease patients in Northern Ireland

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Introduction

Sickle cell disease (SCD) is a group of haemoglobinopathies that contain mutations in the gene encoding the beta subunit of haemoglobin. It is characterised by the presence of sickle haemoglobin. The sickle cell mutation occurs when glutamine is replaced by valine at the sixth position of the beta-globin chain causing sickle-shaped red blood cells, progressive multi-organ failure and an increase in mortality (Kavanagh, P.L et al, 2022).



Complications of SCD

- Patients with SCD usually have a mild to moderate anaemia. Severe anaemia can be due aplastic crisis which is normally caused by an infection with parvovirus B19.
- Acute pain crisis (sickle cell or vaso-occlusive crisis) this is when sickle cells block the blood flow and causes sharp stabbing pain throughout the body.
- Acute chest syndrome is an extremely common cause of hospital admission in SCD patients. RBCs sickle in the blood vessels of the lungs and this can deprive the lungs of oxygen leading to acute lung injury.

Northern Ireland population

Data is limited on the number of people living in NI with SCD. Data taken from the most recent Census (2021) has shown that 3.4% of the population, or 65,600 people, belonged to minority ethnic groups. This is around double the 2011 figure (1.8% – 32,400 people) and four times the 2001 figure (0.8% – 14,300 people)

With this increase in minority ethnic groups, will come an increase in patients requiring treatment/management at BHSCT due to SCD and having this proposed testing available for these patients will positively impact their care.

Red cell Genotyping

Serological techniques for red cell phenotyping remain the gold standard technique for the majority of cases. Nevertheless, these methods still have numerous **technical** and **clinical limitations** that can be difficult to resolve.

Dual population results can occur in red cell phenotyping due the presence of donor cells in the patient's blood and it is impossible to distinguish between donor and patient cells without molecular methods.

Blood group molecular typing is deemed essential in the following cases:

- Transfusion dependent patients: due to the persistence of transfused donor cells (SCD, Thalassemia, Haematological malignancies etc.)
- Patients with warm auto agglutinins and/or positive DAT
- Determining Rh variants
- Testing when rare serological antisera is not available
- Testing to resolve discrepant / weak serology phenotypes

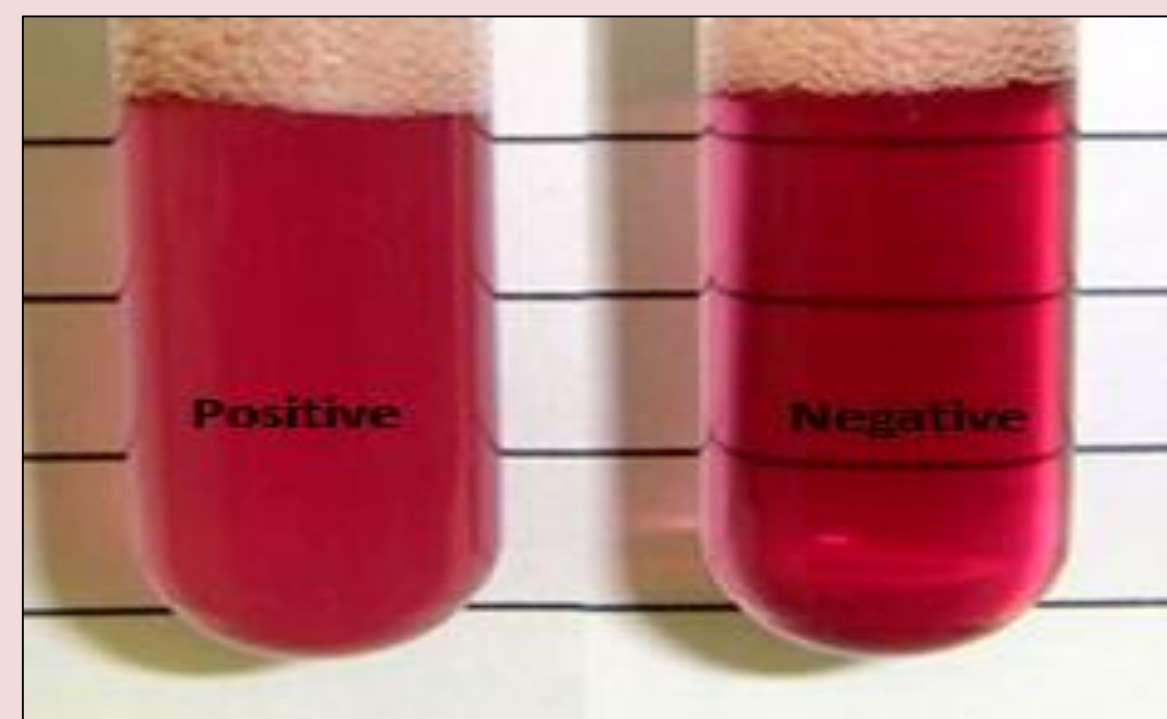
Aim of study

This study aims to investigate the use of Sickledex as a screening method to identify HbS negative donors and to implement a molecular red cell extended genotyping test kit to allow NIBTS to retain an accurate genotyping record when extended RBC phenotyping is not possible, in keeping with guideline recommendations and aid with appropriate selection of blood for transfusion purposes.

Materials and Methods- Sickledex

After assessment of a number of methodologies, the **Sickledex** method of HbS screening was selected due to its low cost and ease of use.

When Sickledex Reagent Powder is combined with Sickledex solubility buffer and a blood sample is added, a patient's blood sample that contains Haemoglobin S will form a cloudy, turbid suspension as seen below;



One hundred samples were tested for HbS using the sickle solubility test (Sickledex), which included; random EDTA donor samples, random donor units, and EDTA samples from known SCD patients. Six samples from known SCD patients were also included within these to verify positive results, which were then compared with capillary electrophoresis confirmation results.

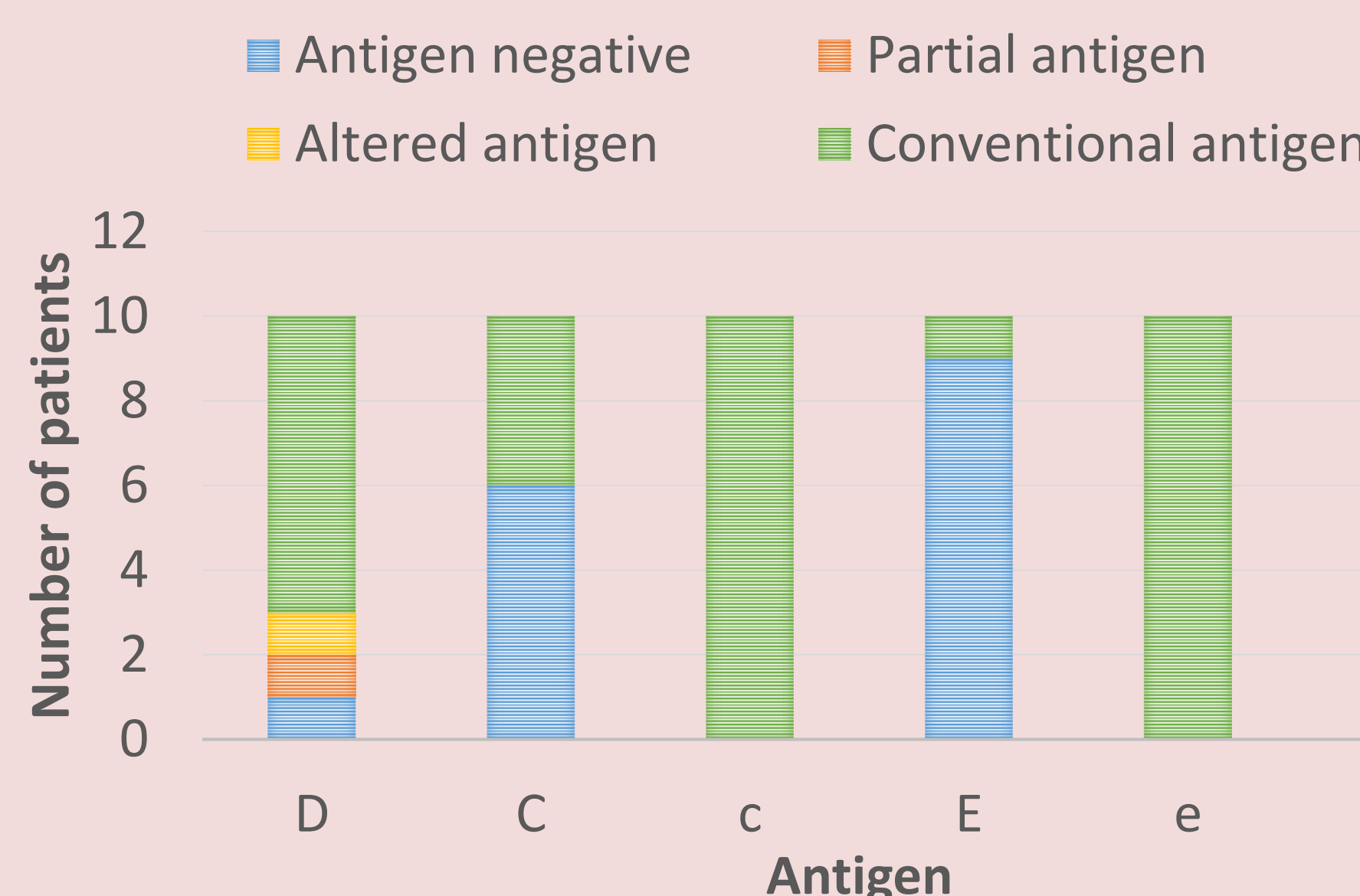
Materials and Methods- Molecular genotyping

Firstly DNA is isolated using the BEXS 12 Bead Extraction System which is an automated system. The RBC-FluoGene vERYfy eXtend plate is the set up with the Pipetting Unit PiU 1.

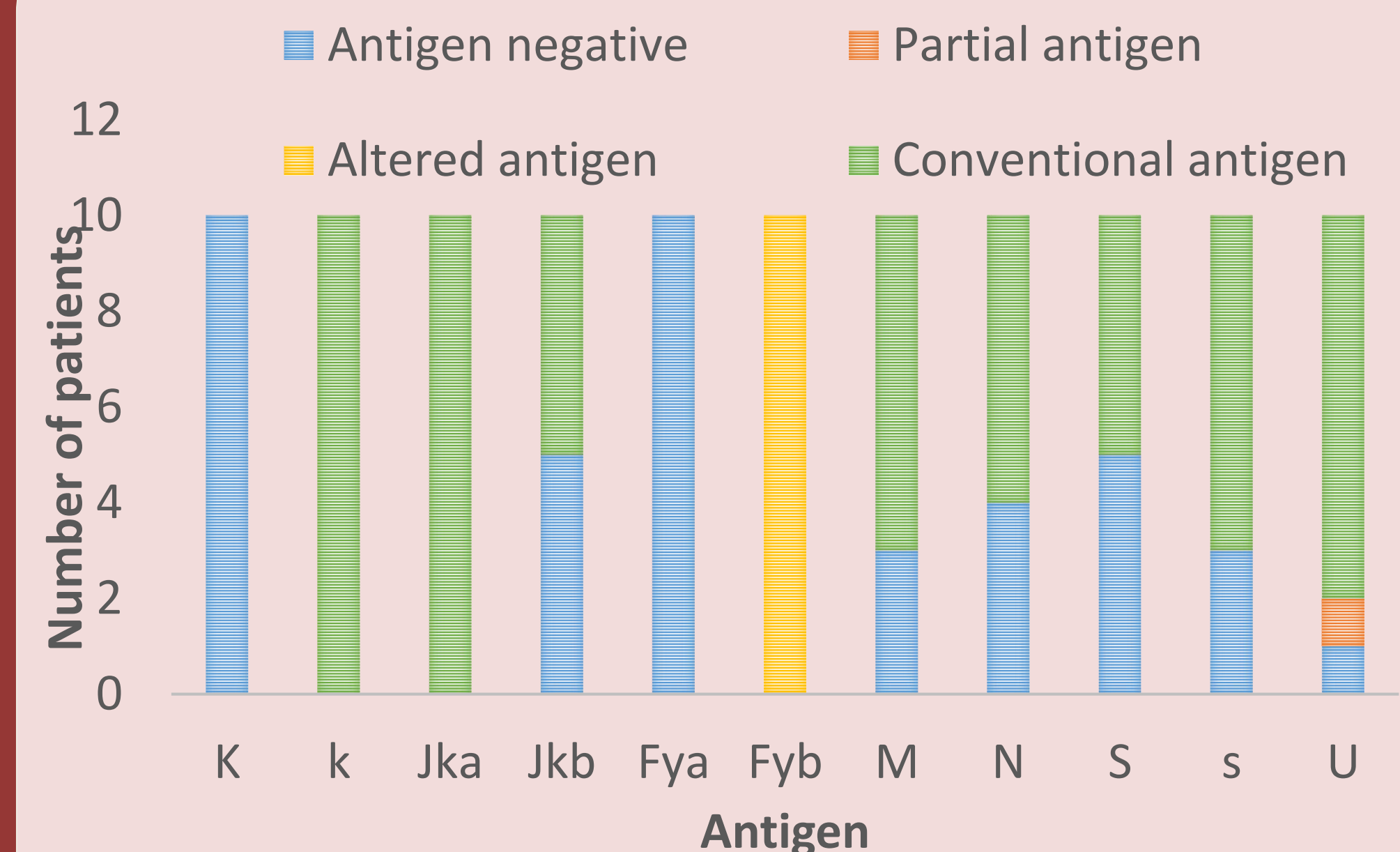
This vERYfy eXtend kit has oligonucleotide mixes that are pre-aliquoted and dried in the PCR plates and this allows specific genetic markers to be amplified. The oligonucleotide mixes contain primers and probes, which are labelled with different fluorescent dyes.

Ten samples testing platform and had red cell genotyping performed from known SCD patients tested using the Inno-train PCR (qPCR) molecular d using the FluoGene vERYfy eXtend kit to accurately determine the genotype of these multiply transfused patients ensuring appropriate blood selection for future transfusions.

Results



Graph showing Rh antigen expression predicted from genotyping that differentiates patients whose red cells express variant, conventional antigens or are antigen negative (n=10 patients)



Graph showing antigen expression of clinically significant antibodies in known SCD patients (n=10 patients)

As expected none of the donation units or donor samples tested positive for HbS. All six of the known SCD patients showed a positive result using Sickledex.

Discussion

A number of important mutations in these SCD patients were identified including absence of U antigen, U variant and GATA-1 mutations. There were also partial antigens identified which had not been previously detected via standard serological methods. These findings are significant for donor and recipient transfusion purposes as they can lead to patients being immunised and can cause difficulties with the provision of blood for further transfusions.

Red cell genotyping for extended antigens makes transfusion safer for SCD patients by reducing the risk of immunisation through detection of numerous variants, distinction of autoantibodies from alloantibodies and predicting clinical significance of antibodies for individual patient groups. This study confirms the importance of implementing extended genotyping into Northern Ireland to aid transfusion decisions along with improving bloodstock management by ensuring blood is prioritised, maintained and available for patients who actually require specific antigen negative blood. This strategy will improve overall red cell usage.

Future Work

Future work based on a larger scale of donor/patient genotyping would be useful to study the clinical impact that RBC genotyping may have in patients with haemoglobinopathies. Future work could include monitoring alloimmunisation rates in these patients to justify Rh matching vs extended matching. It may also be worth considering other transfusion dependent patient groups for genotyping strategies such as patients with aplastic anaemia, myelodysplastic syndrome or thalassemia.

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