

Summer 2019

LifeSouth series on Important Recent Literature

## Is your patient receiving chronic transfusions?

Red cell alloimmunization is a serious problem in chronically transfused patients.

The rate of alloimmunization in the transfused population ranges from 0.5% to 6.5% whereas in patients with Sickle Cell Disease (SCD) ranges from 8% to 36% with an average of 25%.

## The Utility of RBC Molecular Genotyping in Antibody Identification and Transfusion

LifeSouth Immunohematology Reference Laboratories (LS IRLs) use the patient's phenotype to help identify and confirm alloantibodies to red blood cell antigens, to expedite the investigation of samples with multiple antibodies, to prevent further alloimmunization of chronically transfused patients and to improve the safety and survival of transfused red blood cells.



## Phenotyping Methods

Serologic phenotyping can be useful in many antibody investigations, because it is the quickest method of obtaining a partial or an extended phenotype. However, there are cases when it is not possible to obtain a serologic phenotype. These cases include, recent transfusion, positive DAT when IAT only antisera is available, when antisera is not available for the target antigen(s), and when cells are so heavily coated with immunoglobulins that they clump at IS.



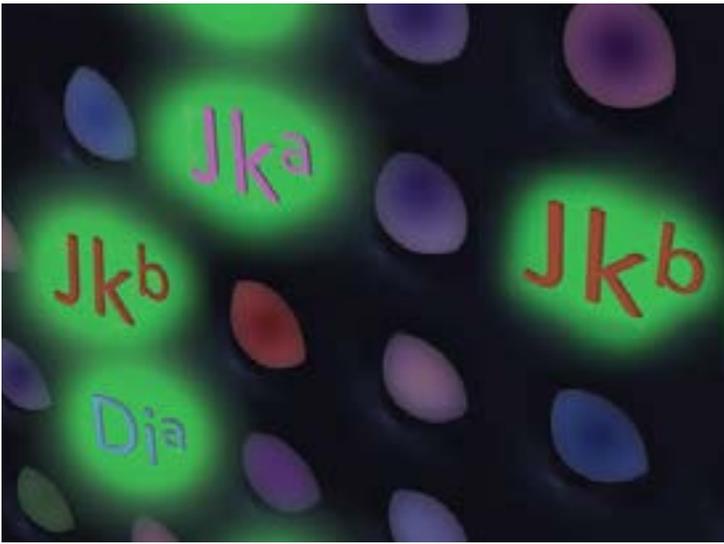
even though they type as Fy(a-) and Fy(b-) by serology. Molecular genotyping can also detect variant genes that produce altered or partial antigens. This information can help explain cases where patients make alloantibodies to antigens that they are positive for by serology, for example, an Rh positive patient who makes alloanti-D (RH1). Last but not least, molecular genotyping can make it possible to find compatible blood at the allele level for patients at risk of making antibodies to most red blood cells, such as patients homozygous for partial e (RH5) (RHCE\*01.20.05 or RHCE\*ceVS.05, 733G and 1006T RH:-10,20,-31) and also negative for E (RH3).

The limitations of molecular genotyping include having an adequate sample from which to extract DNA in order to perform the assay, the amount of time required to complete the assays (several hours), the need for dedicated equipment and software, the risk of contamination by amplified products, mutation of the target allele(s), cost, competency and efficiency in low test volume laboratories. One benefit however, is that it only needs to be performed once and it can be done with a blood sample or buccal swab.

Molecular genotyping can be used in place of serology to predict the red cell phenotype by the detection of Single Nucleotide Polymorphisms (SNPs). A genotype can be performed on a transfused patient because donor white cells in transfused blood are removed by filtration, and the positive DAT does not affect testing.

There are two licensed molecular genotyping assays available in the US (Grifols' IDCORE XT™ and Immucor's BioArray™ HEA) that can predict the phenotype for antigens that have no corresponding licensed antisera in the market, such as V (RH10) and U (MNS5) and for a number of high frequency antigens, such as Dib (DI2) and Kpb (KEL4).

Benefits of molecular genotyping include the ability to detect the presence of a blocking or silencing gene in addition to the gene responsible for the antigen of interest. For example, a mutation in the erythroid promoter GATA-1 silences the expression of Fyb (FY2) antigens on red blood cells (due to a 67T>C point mutation in the GATA-1-binding motif of the FY\*B promoter), but not on other tissues, thus explaining why these individuals do not typically make anti-Fyb or anti-Fy3



## Conclusion

Despite its limitations, LS IRLs find the value of genotyping information to be indispensable in most complex antibody identification investigations with or without accompanying requests for compatible red blood cells. Fortunately, LS IRLs are set up to perform both licensed genotyping assays on a daily basis.

## What the IRL needs:

Our online forms or portal are available to request extended phenotype or genotype for your patient, if transfusion or pregnancy has occurred in the previous 3 months, a genotype could be the test of choice, since serologic phenotype may be less accurate in these situations.

Visit LifeSouth IRL Services website: <http://lifesouth.org/for-hospitals/#lab-irl-services> to access these tools.

*Legend: DAT: Direct Antiglobulin Test; IAT: Indirect Antiglobulin Test; IS: Immediate Spin.*

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