Technical Brief

DNA Microarray for HEA Assay: Red Cell Blood Group Antigen Typing

Background Information

Testing of the red cell phenotype often is required for patients referred to the blood bank for antibody evaluations and selection of phenotype-matched red cell units for transfusion. The standard method of performing the red cell phenotype is by serological assay, using reagent antisera specific for the antigens of interest. However, serological typing is labor-intensive; the antisera are expensive and often in short supply; and the method is not suitable for patients with a positive direct antiglobulin test, those who have been transfused recently; or when a sufficient sample is not available. The HEA assay, a DNA microarray test, provides an alternative to address the limitations of serological testing.

Clinical Indications

An adjunct to standard pre-transfusion testing when a red cell phenotype is needed but serological phenotyping cannot be performed.

Appropriate candidates for the HEA assay include:

1. Recently transfused patients or patients with a positive DAT
2. Patients with sickle cell anemia at high risk for red cell alloimmunization and other frequently transfused patients who are known immune responders
3. Patients with auto-antibodies that interfere with phenotyping and antibody identification
4. Patients at risk for alloimmunization to the V and Js^a antigens, as these antigens are rarely represented on standard antibody panels
5. Selected patients with Fy^b-negative serological phenotype, for evidence of the Fy^b silencing mutation. Patients with this mutation generally do not require Fy^b-negative red cell units for transfusion
6. Patients who need to be typed for high- or low-prevalence antigens for which antisera are not readily available or are unreliable
7. Patients with samples that are insufficient for serological testing

The HEA assay also can be used to test blood donors and donor units to identify antigen-negative units for transfusion.

Interpretation

The HEA assay compares signal intensity on the microarray to the location of specific paired alleles and uses this information to determine the presence or absence of each allele. Based on the allele results, the assay software determines the probable phenotype and lists each allele as:

+ (allele is present).

0 (allele is absent) or

Not reportable (due to problems with signal intensity. Testing may be repeated to obtain valid results.)

The HEA assay may be used for recently transfused patients. However, results should be interpreted cautiously for patients transfused with non-leukoreduced blood, infants, hematopoietic stem cell transplant recipients, immunocompromised patients, and patients with leukopenia (WBC <1000/uL) or leukocytosis (WBC $>10 \times 10^6$/uL).

The HEA assay result is based on the patient's genotype, and occasional discrepancies with the expressed phenotype are expected. Such discrepancies must be investigated.

Limitations of the Assay

1. The HEA assay is not licensed by the FDA. The assay has been validated for use by the Cleveland Clinic Section of Transfusion Medicine. Use for blood product testing is investigational and used as an adjunct to standard testing.
2. The single nucleotide polymorphisms (SNPs) responsible for allelic variants may differ based on the patient's ethnic group, and some variants may not be represented in the assay, giving a false negative result.
3. The assay does not detect all clinically significant antigens as the gene of interest must have been cloned and characterized so that molecular methods may be used for detection.

Methodology

The cloning and characterization of genes encoding several red cell antigens provides the basis for the HEA assay. For many red cell antigens (notably excluding the ABO and D antigens), alleleic differences generally result from SNPs, and detecting

these SNPs provides the basis for determining antigen expression. The HEA assay tests for 24 polymorphisms associated with 32 red cell antigens: C/c, E/e, VS, V, K/k, Js^a/Js^b, Kp^a/Kp^b, Fy^a/Fy^b, GATA (silencing FY), Fy^x [$Fy(b+^w)$], Jk^a/Jk^b, M/N, S/s, silencing S (x2), Lu^a/Lu^b, Do^a/Do^b, Hy+/Hy-, Jo(a+)/Jo(a-), LW^a/LW^b, Di^b, Di^a, Co^a/Co^b, Sc1/Sc2. In addition, the assay tests for the presence of the HbS mutation.

The HEA assay is performed on DNA extracted from EDTA whole blood. The DNA region of interest is amplified by multiplex PCR, processed into single-stranded DNA and hybridized with allele-specific oligonucleotide probes to detect the relevant SNPs. The hybridization reaction is performed on a semiconductor chip mounted on a slide. Each chip contains beads expressing a library of allele-specific oligonucleotide probes matching either the wild-type or mutant allele. The beads are assembled in a 300 x 300um area and contain approximately 4000 beads of multiple spectrally distinguishable types representing the allele-specific probes, and negative and positive control reactions. Hybridization of the test DNA with a matching probe results in probe elongation that is visualized by incorporation of a fluorescent label. An imaging system analyzes the intensities of the fluorescent signals for each SNP, and web-based software interprets the polymorphisms detected and the probable red cell phenotype.

Suggested Reading

1. Hashmi G *et al*. A flexible array format for large-scale, rapid blood group DNA typing. *Transfusion*. 2005;45:680.
2. Klapper E *et al*. Toward extended phenotype matching: a new operational paradigm for the transfusion service. *Transfusion*. 2010;50:536.
3. Reid ME *et al*. DNA from blood samples can be used to genotype patients who have recently received a transfusion. *Transfusion*. 2000;40:48.
4. Ribeiro KR *et al*. DNA array analysis for red blood cell antigens facilitates the transfusion support with antigen-matched blood in patients with sickle cell disease. *Vox Sang*. 2009;97:147.
5. Rozman P *et al*. Differentiation of autologous ABO, RHD, RHCE, KEL, JK, and FY blood group genotypes by analysis of peripheral blood samples of patients who have received multiple transfusions. *Transfusion*. 2000;40:936.
6. Wenk RE, Chiafari FA. DNA typing of recipient blood after massive transfusion. *Transfusion*. 1997;37:1108.

Test Overview

Test Name	HEA Assay
Reference Range	n/a
Specimen Requirements	EDTA whole blood (pink-top) tube, refrigerated
Special Information	Blood bank requirements apply for sample collection and labeling, and phlebotomist identification
Stability	Refrigerated: 7 days
CPT Code	83891; 83892 (x2); 83900; 83901 (x22)
Billing Code	87880

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