PreciseType™ HEA Test

Molecular Technology Personalizes Donor/Patient Compatibility

The first in the line of Immucor PreciseType personalized-medicine diagnostics, the PreciseType HEA (human erythrocyte antigen) test rapidly and accurately predicts blood compatibility between donors and patients to help prevent mismatches that can cause a serious immune reaction (alloimmunization) in patients. Each year, almost five million Americans need a blood transfusion¹. Blood incompatibility remains a significant problem²-⁵ with lifelong consequences that adds to the burden of healthcare delivery and may result in life-threatening delays in care. In standard cases, two to six percent of transfusion patients are alloimmunized. In certain populations that require multiple transfusions because of blood disorders, such as sickle cell disease or thalassemia, the alloimmunization rate reaches as high as 36 percent because the donor blood was not a close enough match to their own⁶. Sickle cell disease and thalassemia are inherited blood disorders in which the body makes an abnormal form of hemoglobin, the protein in red blood cells (RBCs) that carries oxygen.

Knowledge = Power to Improve Health Outcomes

Medical researchers have made great strides over the last two decades in understanding the genetics behind transfusion reactions and the importance of a precise phenotype match. Scientists have sequenced the genes encoding blood-group systems and have identified the genetic mutations associated with the development of specific antigens. If an antigen-negative patient receives blood from an antigen-positive donor, it could trigger an immune reaction, where the blood recipient’s immune system develops antibodies that can attack and reject the donor RBCs. Immucor’s PreciseType HEA test leverages the latest advances in medical research and molecular technology. It is the first FDA-approved test that uncovers the specific gene variants associated with RBC antigens to provide the closest match now possible between donor and recipient in a single test. The PreciseType HEA test uses 24 known gene mutations to identify 35 red blood-cell antigens and three phenotypic variants from 11 blood groups simultaneously.
Personalized Medicine Begins with an Accurate RBC Genotype/Phenotype Profile

With today’s dual focus on improving health outcomes and lowering health-care costs, preventing alloimmunization (development of antibodies that attack antigens seen as “foreign” by the immune system) is the ultimate goal in transfusion medicine. Accordingly, a best practice for the hospital or transfusion center is to create a patient phenotype profile with the Precise-Type test before a patient receives his or her first-ever transfusion. Donor blood would be similarly profiled and catalogued, with rare types saved for special cases, to facilitate selection of only those units that are a molecular match between donor and recipient. If the patient has already received multiple transfusions, a PreciseType molecular profile remains a critical tool to identify the best-matched donor blood. A limitation of traditional serology testing is that it can be difficult to interpret for some months after a transfusion when donor red cells remain along with the patient’s own in the sample. By contrast, the PreciseType assay is a molecular-level test—multiple RBC populations do not compromise interpretability or accuracy.

Sample to Result with the PreciseType HEA Test

Sample Preparation

The PreciseType process begins with extraction and isolation of deoxyribonucleic acid (DNA) from a sample of whole blood.

Manufacturing and Molecular-level Precision

Molecular-level precision starts with tiny beads, which are just three microns across—about one-tenth the diameter of a human hair. Each bead is stained with a distinct blend of ultra-violet, blue, and green fluorescent dyes that give it a unique color signature. Each bead also contains more than one million copies of a particular “blood-group specific probe” (allele-specific oligonucleotide) that are bound to its surface. Different colored beads carry different probes. The probes work like nanosized docking stations customized to detect the presence of 24 known gene mutations (antigen-specific polymorphisms) that determine the RBC antigens. About 4,000 differently colored beads (with about 50 or 60 copies of each color) are affixed randomly to the surface of a silicon chip to form a single BeadChip. The fully assembled BeadChips are then bonded to a glass slide or a plate. The two available formats are designed to increase testing efficiency in labs with different throughputs. The slide format allows eight, and the plate format allows 96 simultaneous evaluations of patient and/or donor samples. Each slide and plate is barcoded with a unique ID.
A chain reaction makes thousands to millions of copies of multiple segments of DNA so the mutations of interest that reside within the amplified segments can be analyzed.

Following PCR, the excess reactants are deactivated enzymatically. Enzymes then separate the double-stranded DNA helix into two single strands, digesting one, and leaving the other intact. The remaining single strand of the original DNA segment is combined with the signal-development reagent and pipetted onto the surface of each BeadChip, which are then placed in a hybridizing oven.

Unlocking Assay Results
As part of the manufacturing process, a bead map (i.e. location of the colored beads carrying the blood-group-specific probes) is established for each BeadChip. The bead-map key for all BeadChips manufactured in a lot is stored on a CD, included with each test kit. After running the assay, this unique beadmap key is required to analyze assay results.

What’s in the Kit?
The PreciseType HEA Kit includes supplies to perform 96 tests in two available formats: 12 eight-chip slides or one 96-chip microplate. The kit includes two boxes.

The first box contains all the required PCR, post-PCR, and signal-development reagents along with a negative control necessary for the test.

The second box includes the barcoded BeadChip slides or plate, along with a disk that holds the chip-specific bead-map key, necessary to analyze results after running the assay.
In the warm, humid environment of the oven, DNA sequences hybridize to the blood-group-specific probes on the BeadChip. If the DNA sequence in the sample matches perfectly with the probe sequence on the BeadChip, the probe will be extended through the incorporation of fluorescently tagged nucleotides.

**STEP THREE: Hybridization**

If there is not a precise match, elongation does not occur.

**Limitations of Serology**

At the March 2014 meeting of the FDA Blood Products Advisory Committee, Orieji Illoh, MD,OBRR, deputy director in the FDA’s Office of Blood Research and Review, Division of Blood Applications, outlined some of the limitations of serology in her introductory statement. Dr. Illoh said that in many cases, there are no licensed antisera to most rare antigens and when licensed antisera do exist, availability can sometimes be limited. She added that routine screening of large numbers of donor units for all potential antigens is hampered because serological processes are time consuming and resource intensive. Dr. Illoh also highlighted the difficulty in determining antigen specificity in a person who has had recent transfusions, given the multiple populations of red cells that remain in the blood sample. In addition, she noted that the presence of an auto-antibody (a positive DAT) can also affect serology results, as could differences in reagent reactivity.
STEP FOUR: Analysis & Results

Next, the Beadchips are imaged with Immucor’s AIS (Array Imaging System), a specially equipped fluorescence microscope-based reader. The reader detects where there is a fluorescent signal in the assay image, and then determines which particular blood-group-specific probe was associated with the signal. Immucor’s proprietary BASIS™ software suite then uses proprietary algorithms to interpret the results, and generates a report detailing the blood group genotype and phenotype.

Hands-on time from multiplex PCR through results reporting is about one-and-a-half hours

Not including sample preparation, running the PreciseType HEA assay from multiplex PCR through results reporting takes approximately six hours for up to 96 samples. This allows for completion of the assay within a single work shift.

Becoming the New Standard of Care in Molecular Red Blood Cell Matching

The use of serology to determine red blood cell antigen phenotypes has been very successful in providing safer transfusions. However, there are limitations (see sidebar on page four), which are addressed by the use of molecular technology, like the Immucor PreciseType assay. The company introduced a research-use-only version of its molecular HEA assay in 2005. Since then, more than 80 sites have conducted approximately 700,000 tests, including many of the largest donor centers and most-prominent medical centers around the globe. The test received a CE IVD mark in May of 2010, and currently 35 centers are using the technology outside of the U.S., primarily in Europe. With its approval of the PreciseType HEA test, the FDA has ushered in a new era—where molecular blood donor/recipient matching can finally become the standard of care.
References


To learn more, call 855.IMMUCOR (855.466.8267), or visit www.immucor.com.