

## SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

### I. GENERAL INFORMATION

*Device Trade Name:* Immucor PreciseType™ Human Erythrocyte Antigen Molecular BeadChip Test

*Device Procode:* PEP, Molecular Erythrocyte Typing Test (Hematology Panel).

*Applicant's Name and Address:* BioArray Solutions, an Immucor Company (BAS)  
35 Technology Drive, Suite 100  
Warren, NJ 07059

*Premarket Approval Application (PMA) Number:* BP130026

*Date(s) of Panel Recommendation:* March 18, 2014

*Office's Signatory Authority:* Jay S. Epstein, M.D.  
Director, OBRR/CBER

- I concur with the summary review.**
- I concur with the summary review and include a separate review to add further analysis.**
- I do not concur with the summary review and include a separate review.**

*Date of FDA Notice of Approval:*

## II. INDICATIONS FOR USE

The following is the intended use statement for the Immucor PreciseType™ (PreciseType) Human Erythrocyte Antigen Molecular BeadChip Test:

The PreciseType HEA Molecular BeadChip Test is an *in vitro* diagnostic test intended for the molecular determination of allelic variants that predict erythrocyte antigen phenotypes in the **Rh** (C [RH2], c [RH4], E [RH3], e [RH5], V [RH10], VS [RH20]), **Kell** (K [KEL1], k [KEL2], Kpa [KEL3], Kpb [KEL4], Jsa [KEL6], Jsb [KEL7]), **Duffy** (Fya [FY1], Fyb [FY2], GATA [FY-2], Fyx [FY2W]), **Kidd** (Jka [JK1], Jkb [JK2]), **MNS** (M [MNS1], N [MNS2], S [MNS3], s [MNS4], Uvar [MNS-3,5W], Uneg [MNS-3,-4,-5]), **Lutheran** (Lua [LU1], Lub [LU2]), **Dombrock** (Doa [DO1], Dob [DO2], Hy [DO4], Joa [DO5]), **Landsteiner-Wiener** (LWa [LW5], LWb [LW7]), **Diego** (Dia [DI1], Dib [DI2]), **Colton** (Coa [CO1], Cob [CO2]), and **Scianna** (Sc1[SC1], Sc2 [SC2]) blood group systems in human genomic DNA. The test also detects a mutation in the Beta Globin gene. The results from this mutation detection are not intended for diagnosis of Sickle Cell Disease.

## III. DEVICE DESCRIPTION

PreciseType, version 1.2, is a deoxyribonucleic acid (DNA) microarray assay that utilizes polymerase chain reaction (PCR) amplification of genomic DNA coupled with an elongation mediated multiplexed analysis of polymorphisms (eMAP) platform to determine allelic variants of human erythrocyte antigens (HEA). PreciseType consists of the PreciseType assay kit, the Array Imaging System instrument, and the BioArray Information Systems Interpretive Software.

### a. Precise Type Assay Kit

The kit contains the following reagents and components:

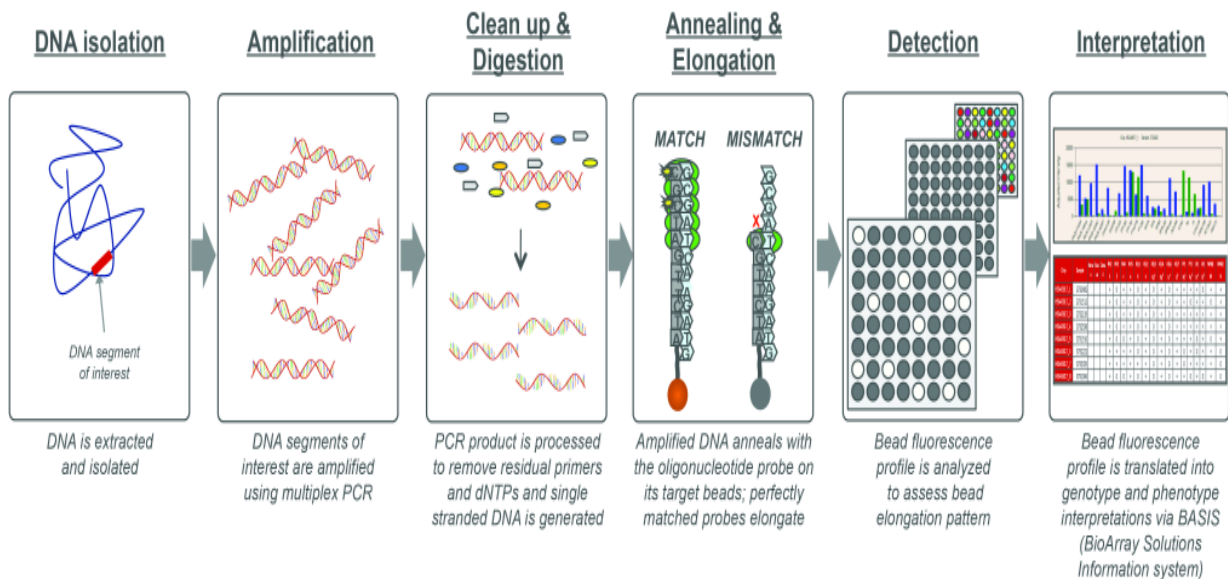
- HEA 1.2 PCR Mix
  - Clean-up Reagent
  - Lambda Exonuclease
  - HotStar Taq DNA Polymerase
  - eMAP Elongation Mix
  - Negative Control
  - 8 or 96 BeadChip Carriers (eight bead chips on a slide or 96 bead chips on a plate)
  - HEA Carrier Data
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The PreciseType bead chip is a planar array with approximately 4,000 fluorescently-encoded 3.2 µm beads. Different oligonucleotide probes specific for nucleotide polymorphisms in HEAs are covalently attached to spectrally distinguishable beads, each with a different and unique fluorescent signature. Each fluorescently-encoded bead contains a unique blend of three primary fluorescent dyes (Ultraviolet, Blue and Green), that generates a distinct fluorescent signature. These unique fluorescent signatures are mapped to the corresponding probes during the manufacturing process and the information is included in the HEA carrier data. Every chip contains approximately -----(b)(4)----- beads for each oligonucleotide probe. The beads for all specificities are randomly located on the chips; however their exact locations are established at the time of chip manufacturing and recorded as a bead map that is unique for each chip array.

The bead map (the location of each bead in the chip array) for each chip and the master lot signal intensity thresholds are stored on a CD-ROM included with each carrier kit. End users transfer these data to their computers where the sample image files from the Array Imaging System (AIS) are merged with the manufacturing data on the bead map and the genotype determinations are made based on the thresholds determined for each lot.

The assay consists of five steps (see Figure 1), followed by genotype analysis and phenotype determination using BAS proprietary BASIS™ software. The process begins with extraction of DNA from whole blood, followed by multiplex polymerase chain reaction (mPCR) amplification of genomic regions of interest simultaneously generating multiple amplicons. Once the mPCR is complete, residual oligonucleotide primers and deoxyribonucleotide triphosphates (dNTPs) from the reaction are digested using ExoSAP (Exonuclease I and Recombinant Shrimp Alkaline Phosphatase) in the clean-up step, which is then followed by Lambda Exonuclease digestion to generate single-stranded DNA (ssDNA) targets. The ssDNA targets are then applied to the PreciseType bead chip. While the ssDNA targets incubate on the bead chip surface, only probes with complements to the ssDNA targets hybridize and elongate, incorporating a fluorescently-labeled deoxycytidine triphosphate (dCTP). The elongation products on the bead chip surface are then detected using the BAS AIS 400 instrument.

**Figure 1: Summary of PreciseType Assay and Interpretation Steps**



**b. Assay Controls (external)**

The end user must use two positive controls and one negative control to determine run validity.

**Negative Control:**

The Negative Control is vial PCR grade water purchased from ----(b)(4)----- and relabeled with a BAS label. The Negative Control is tested upon receipt and is stored at minus 20-80 °C with an expiry dating assigned by the supplier. A Negative Control is supplied with each PreciseType kit and is required for each run.

**Positive Control:**

The following positive controls, purchased separately from the PreciseType kit by the end-user (BeadCheck HEA Positive Control kit), are required for each run: HEA BeadCheck Reference Panel-A and Reference Panel-B (HEA BeadCheck). The BeadCheck HEA Positive Control kit was submitted as an abbreviated 510(k) and cleared under BK130050.

The HEA BeadCheck kit consists of AA and BB synthetic plasmid pools designed to demonstrate the assay will detect all 'A' and all 'B' allelic forms of each genetic marker in PreciseType. The HEA BeadCheck Kit is not intended to monitor the

DNA extraction step of the PreciseType test. The HEA BeadCheck Kit (two positive controls) and the negative control supplied with PreciseType are required to be used for each test run and for each lot used within a run if more than one lot is needed to accommodate the sample size.

**c. Array Imaging System (AIS) Instrument**

The AIS instrument includes a microscope, -----(b)(4)----- control box with automatic stage, focus motor and filter wheel, light source, camera, computer and a software package including the Array Imaging System Reader (AISR). The hardware components acquire the images necessary for analyzing the PreciseType arrays. The AISR software program controls the operation of the microscope including exposure time, auto positioning and auto focus. The AIS instrument captures the fluorescent signal from individual beads in an array image, determines the identity of each bead by its position in the array, and reports the average signal intensity, coefficient of variation of the intensities, and number of beads measured for each type of probe. This information is transferred to the BASIS interpretive software. The AIS instrument was submitted as an abbreviated 510(k) and cleared under BK130051.

**d. BioArray Solutions Information Systems (BASIS) Interpretive Software (version 4.0.13)**

BASIS is a suite of software used to provide secure access for users to store, analyze, and manage PreciseType bead chip related information. BASIS authentications are sent via Hypertext Transfer Protocol Secure (HTTPS), a communications protocol for secure communication over a computer network. An encrypted authentication cookie is used during the user's session. Both HTTPS and digital signing are used to prevent any tampering at the client site and cyberspace. The BASIS AM Global server resides in a secure location with continuous, round-the-clock monitoring services. Authorized users have a login ID and password for access and use of PreciseType web applications.

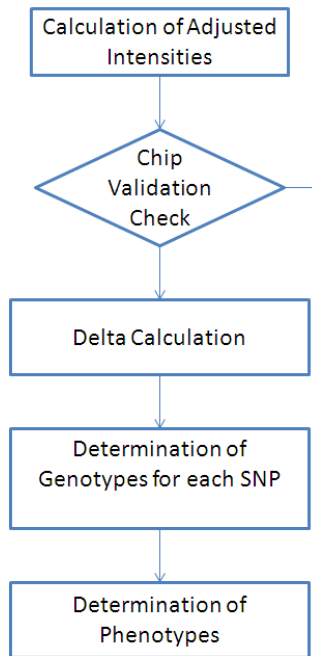
BASIS software is designed for use with the AIS computer. BASIS links the sample identification of each specimen to the individual PreciseType bead chip read on the AIS instrument. After this link is established, the BASIS software generates a report showing the results for each individual specimen on which the assay is performed.

The end user is linked to the BASIS interpretive software either locally (installed on the AIS computer), or globally, through the web (BASIS 4G Global). The signal intensity thresholds information for each master kit lot is downloaded by BAS to the BASIS 4G Global server and is also provided in CD format in each kit. The thresholds for each antigen pair are established during manufacturing at BAS to be

able to discriminate between the different genotypes for each HEA allele i.e., homozygous wild type, homozygous mutation and heterozygous samples.

The BASIS information flow for a DNA sample outlined in Figure 2:

**Figure 2. BASIS Information Flow**



BASIS 4.0.13 was evaluated by FDA using Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices, issued on May 11, 2005. The review of the software included an evaluation of BioArray risk management activities as they related to BASIS 4.0.13 and a review of their validation activities, including but not limited to Software Description, Device Hazard Analysis, Software Requirements Specification, Software Design Specification, Traceability Analysis, Software Development, Revision Level History, and Unresolved Anomalies. In addition, the genotype and phenotype assignments were compared to the published scientific literature submitted by BioArray.

**e. Genotype Determination**

The assay signal intensities produced for each single nucleotide polymorphism (SNP) by the corresponding pair of elongation probes provide the basis for allele discrimination. The BASIS interpretive software imports the raw intensity output

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from the AIS instrument, assesses the validity of the internal negative control probes, and generates assay results. The background signal, as measured by the internal negative control probe, is subtracted from each probe signal. An internal negative control probe is included for each dye (Ultraviolet, Blue and Green) to permit background subtraction of the fluorescence contributed by the internal stain dye in each microsphere.

The delta value (discrimination ratio) is calculated  $\{(Intensity\ of\ Probe\ A - Intensity\ of\ Probe\ B) / (Intensity\ of\ Probe\ A + Intensity\ of\ Probe\ B)\}$  for each probe pair using the background corrected intensities and is compared to lot specific thresholds. As stated above, lot specific thresholds are provided to the end user on the CD provided with each kit. BAS also downloads onto a server the lot specific threshold for end users that use the BASIS 4G global server for genotype calculations and phenotype conversions. Depending upon the intensity of each probe, probe pair delta values (discrimination ratio) can be classified into several regions which correspond to genotypes. In general, these regions correspond to homozygous (AA), heterozygous (AB), and homozygous (BB) genotypes, or an indeterminate result (XX).

#### f. Genotype to Phenotype Conversion

A lookup table that correlates the genotyping results to the reported antigen expression state is used for the genotype-to-phenotype conversion. The general rule for single SNP phenotype calculation is described in Table 1.

**Table 1. Genotype to Phenotype Conversion**

GENOTYPE	PHENOTYPE
AA (only A allele detected)	+0 Examples: E positive, e negative Lu <sup>a</sup> positive, Lu <sup>b</sup> negative
AB (both A and B alleles detected)	++ Examples: E positive, e positive Lu <sup>a</sup> positive, Lu <sup>b</sup> positive
BB (only B allele detected)	0+ Examples: E negative, e positive Lu <sup>a</sup> negative, Lu <sup>b</sup> positive
XX (Indeterminate Call for both alleles)	ICIC (Indeterminate Call)* No type determined

AX (A allele detected and indeterminate call detected)	+IC  No type determined
BX (B allele detected and indeterminate call detected)	IC+  No type determined

Some of the antigen systems present on the PreciseType are more complex and do not follow the pattern mentioned above. For example:

- The U antigen, is inferred through the presence of or absence of three single nucleotide polymorphisms (SNPs) on one gene. The presence of any one of these SNPs represents the presence of U in a typical or variant form; the absence of all three (determined by the absence of signal for all three probes) introduces a fourth genotype, the U (-) phenotype.
- Some negative phenotypes are inferred through genotype analysis by the detection of known silencing polymorphisms. These polymorphisms are linked with a particular antigen and can be used to determine if the antigen is present in the phenotype. In this case, the status of the silencing probe pair, which is separate from the probe pair that directly detects the antigen's genetically coded polymorphism, determines the presence of an antigen (ex. Duffy antigen).
- The determination of the RhC phenotype requires the presence of a SNP as well as a linked insertion elsewhere in the RhC gene sequence.

The genotype and phenotype assignments are all based on published scientific literature. Genotype and phenotype calculations are performed by the BASIS interpretive software.

#### g. Description of Expected Results

##### Quality Control

The determination of the run and sample results validity is performed by the BASIS software. For samples that are part of an invalid run or have invalid phenotype(s) (sample results invalid), all antigen phenotype results are reported as No Type Determined (NTD).

**Run Validity:** Two positive and one negative control as supplied, are required for each run. The results for all controls must meet the Run Validity criteria. If any



one of the controls does not meet any one criterion, the run is invalid and must be repeated.

Less than thirty two (32) Low Signal (LS) results in the phenotype report for the Negative Control sample indicate possible contamination by genomic DNA in a quantity that may impact test results. When this occurs, all sample results in the run are invalid.

The phenotype pattern of the two positive control samples must match the expected phenotype pattern. If the controls do not meet the test criteria, all sample results in the run are invalid.

Interpretation of validity of the Positive and Negative Controls is summarized in Table 2.

**Table 2: Run Validity Criteria**

<b>Control</b>	<b>BASIS Analysis</b>	<b>Result Reported</b>	<b>Interpretation</b>
<b>Negative</b>	<b>LS <math>\geq</math> 32</b>	<b>Valid NC</b>	<b>Valid Negative Control</b>
<b>Negative</b>	<b>LS &lt; 32</b>	<b>Invalid NC</b>	<b>Invalid Negative Control; No results reported for all samples in the run</b>
<b>Positive</b>	<b>Phenotype Pattern matches pattern expected for the two positive controls from the BeadCheck kit</b>	<b>Valid HEA Ref-pA and Valid HEA Ref-pB</b>	<b>Valid Positive Control</b>
<b>Positive</b>	<b>Phenotype Pattern does not match expected pattern for either one or both of the two positive controls from the BeadCheck kit</b>	<b>Invalid HEA Ref-pA and/or Invalid HEA Ref-pB</b>	<b>Invalid Positive Control; No results reported for all samples in the run</b>

**Sample Results Validity:** For sample results to be valid, the phenotype results for all antigens must be valid. Table 3 describes the causes of invalid sample results. If any antigen phenotype has an Indeterminate Call (IC) or Low Signal (LS) result, the sample results are invalid, except for S and s where LS is an

expected phenotype in conjunction with U negative results. If the sample has High Background (HB) or High Coefficient of Variation (CV) status the sample results are invalid.

**Table 3: Causes for Invalid Sample Result**

CAUSE	INTERPRETATION
IC $\geq$ 1	Indeterminate Call
LS $\geq$ 1	Low Signal
HB	High Background
CV	High Coefficient of Variance

### Analysis of Results

This is a qualitative test. BASIS computes BeadChip array signal intensity data on each oligonucleotide to determine the presence or absence of each allele or the genotype result. The genotype results are then utilized to compute the predicted antigen phenotype results.

All calculations are performed by the HEA analysis software. Expected genotype results are shown in Table 4 below.

For samples that are part of an invalid run or have invalid genotype(s) themselves (sample invalid), all genotype results are reported as NTD.

For samples with valid results, the expected phenotype results are shown below in Table 5.

**Table 4: Expected Genotype Results**

Result Reported	Interpretation
AA	Homozygous for A
AB	Heterozygous
BB	Homozygous for B
IC	Indeterminate call on A and B

**Table 5: Expected Phenotype Results**

<b>Result Reported</b>	<b>Interpretation</b>
+	Positive
0	Negative
(+)*	Possible (C)ce <sup>s</sup> haplotype
(0)*	Fy <sup>b</sup> variant
PV	Possible Variant
Var	U variant (S silencing mutation)
W	Fy <sup>b</sup> Weak
++	HbS homozygous

#### IV. WARNING, PRECAUTIONS, And LIMITATIONS

##### Warnings and Precautions

1. In samples of human origin, there is still a potential risk of infection even after DNA extraction. Handle samples using universal precautions. Use appropriate personal protective equipment throughout the test procedure, including gloves and lab coat. In the event of contact with eyes, rinse immediately with plenty of water and seek medical advice.
2. Never pipette by mouth. Avoid contact of reagents and specimens with skin and mucous membranes.
3. Dispose of used materials in accordance with the Institution's and or local regulations for disposal of potentially bio-hazardous materials. Spillage of potentially infectious material should be cleaned and disposed of immediately in accordance with the institution's policy and procedure for the handling and disposal of potentially biohazardous materials.
4. PCR technology is susceptible to contamination, especially from its own product. Aerosols of PCR amplicons that are generated during the post-PCR steps are a frequent source of contamination. Thus care should be taken to prevent excessive splashing and generation of aerosols. Standard PCR laboratory measures that include wiping of work surfaces before processing or preparing PCR samples with a freshly prepared 10% bleach (or equivalent), use of ultraviolet (UV) light in hoods or biosafety cabinets in between use, space and time separation of pre- and post-PCR activities, use of aliquoted PCR reagents, use of positive and negative controls, etc. should also be followed during the use of the kit. Use of consistent, careful technique coupled with liberal incorporation and monitoring of controls will ensure a vigilant, proactive approach to control and monitoring of PCR contamination.

5. It is required that operators participate in the PreciseType HEA Molecular BeadChip Test Training Program prior to performing this assay in order to assure consistent and accurate test results.
  6. Laboratories should validate their own cleaning procedures.
  7. Contamination of reagents or specimens may cause erroneous results; therefore, care should be taken to avoid contaminating this product during use. Do not use contaminated reagents.
  8. Microbial contamination of reagents or specimens may lead to incorrect results.
  9. Use the kit liquids and BeadChip carriers as supplied. Dilution or alteration may generate erroneous results.
  10. Do not mix reagents or BeadChip carriers between different lots.
  11. Do not use leaking or unlabeled vials.
  12. Previously frozen samples or reagents should be thoroughly mixed and then centrifuged after thawing prior to testing. Avoid generating foam and bubbles in the samples.
  13. Keep all enzymes and master mixes on ice or cryo block (2 - 8°C) during use.
  14. Ensure proper sample tube sealing prior to amplification to prevent evaporation.
  15. Due to inherent differences in the mechanisms of thermal cycler performance, variation in results can occur when set thermal profiles are transferred between different makes and models of thermal cycler instruments. In some cases, reaction specificity and sensitivity can be compromised, leading to the false interpretation and reporting of data. Alternate thermal cyclers and profiles must be validated by the user.
  16. Samples must remain in the BeadChip reaction well during testing.
  17. Incubation times or temperatures other than those specified may give erroneous results.
  18. On the day of use, prior to using the AIS 400C the Exposure Test Carrier (ETC) procedure must be performed to verify performance of the AIS. If the Exposure Test fails, please contact customer service for appropriate instructions. (See AIS User Manual 190-20185)
  19. Deviation from the recommended directions for use may result in less than optimal product performance. Depending upon the nature and severity of the deviation assay failure (individual sample as well as run failures) and/or erroneous results may occur. For example, use of insufficient/inactive Clean-up Reagent in the assay may result in high incidence of false Kp(a)+ calls.
  20. The results from the mutation in the Beta Globin gene are not intended for diagnosis of Sickle Cell Disease.
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**Limitations**

1. False negative and/or invalid results may be generated when unanticipated rare mutation(s) affecting the primer or probe binding cause allele and/or amplicon dropout.
    - Presence of RH hybrids and variant mutations in exons 2, 5 and 7 as well as introns 1, 2, 4, 5, 6, and 7 of the RHCE gene can interfere with the detection of E/e and C/c antigens. Mutations in RHCE gene leading to the ceMO phenotype, which expresses as a weak Rhe, can cause a direct suppression of the Rhe probe and may cause an invalid or false negative result. In select populations, such as the Afro-Caribbean Sickle Cell Disease patients, the prevalence for ceMO phenotype has been reported to be up to 2 %.
    - Presence of a rare +3g>a change in intron 5 of GYPB interferes with the detection of the S antigen and may lead to a false negative typing of the S antigen.
    - The HgbS mutation in the Beta Globin gene should not be used for determination of Sickle Cell Disease. Presence of HbSC disease interferes with the detection of the HgbS mutation in the Beta Globin gene mutation and may result in invalid or inaccurate HbS phenotype call (HbS (++) instead of HbS (+)). In the United States, HbSC disease has a prevalence of 0.017% among African Americans. Presence of some beta thalassemia disorders may interfere with the detection of the HgbS mutation in the Beta Globin gene and may result in an invalid HbS phenotype call.
    - Presence of Mit+(GYPB 161G>A) mutation may result in an invalid or false negative typing of the S antigen. The mutation has a prevalence of 0.1% in western Europeans.
    - Presence of a GYPB mutation (c. 137-8C>T) may result in an invalid or false negative typing of the S antigen.
  2. False positive and/or invalid results may be generated in rare cases where a sample contains examples of molecular events that affect the blood group antigen expression and phenotypes, (such as, DNA sequence variations including premature stop codon, SNP leading to missense change in amino acid, hybrid genes, modifying genes; changes at the RNA transcription level including alternative splicing; reduced protein expression, etc.) and the nucleotide changes associated with these events are not explicitly monitored by the assay. Known phenotypes are Knull, JKnull (JKnull has a prevalence of up to 9% among Polynesians), Rhnull, Rh hybrids, Kmod, Co(a-,b-), In(Lu), Lu(a-,b-) and GP hybrids. Presence of a c.179\_180del (Ser60fs) mutation linked with the Fy(b) allele may change the Fy(b) antigen expression and lead to a false positive call.
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3. The BASIS software is not designed to convert all genotype/allele combinations into phenotype calls. For example, if allele combinations that have not been widely reported in the literature are encountered, the software will display a Possible Variant (PV) call.
4. The HEA test utilizes two point mutations, 733C>G(L245V) in exon5 and 1006G>T(G336C) in exon7 of the RHCE gene to predict the V and VS antigen phenotypes.

The genotype to phenotype prediction conversion rules employed by the HEA test utilizes the established fact that the absence of the two mutations are correlated with the absence of V and VS antigens and that the presence of the mutations lead to antigen expression.

5. In the HEA test, the presence/absence of the RhC antigen is reported based on three polymorphisms 307C>T(P103S) in Exon 2, 733C>G (L245V) in Exon 5, 1006G>T (G336C) in Exon 7 and the presence/absence of 109 bp–insert in Intron 2 of the RHCE gene.

The (+)\* call on the RHC antigen implies the possible presence of altered C antigen encoded by the (C)ces haplotype. The (C)ce<sup>s</sup> haplotype is comprised of:

- i) A hybrid RHD-CE-D allele of the RHD gene, and
- ii) ce<sup>s</sup> allele of the RHCE gene

The (C)ce<sup>s</sup> haplotype produces weak C, normal c, weak e called e<sup>s</sup>, and VS (RH20).

6. The U antigen (located on the GPB protein), is not polymorphic by itself. The expression of the U antigen is governed by changes which affect the expression of the S antigens. Specifically, the S-s- phenotype is known to be associated with the absence or weak expression of the U antigen. The HEA test monitors three mutations which inform the S-s- phenotype and can support a determination of U(var) and U(neg) phenotype. Occasionally due to non-specific residual intensities on the probes governing the silencing of S/s antigen, a U(neg) phenotype call may not be made even if the phenotype call is S-s-.
7. The Fy<sup>x</sup> allele encodes an amino acid change which causes Fy(b+w) phenotype with varying degrees of weakened Fy<sup>b</sup> antigen. Licensed serological anti-Fy<sup>b</sup> reagents may not always react with such a weakened Fy<sup>b</sup> antigen.

## **V. ALTERNATIVE PRACTICES AND PROCEDURES**

Currently, the standard methodology for the determination of human erythrocyte antigens is by hemagglutination, which is performed using anti-sera with known antigen specificity. FDA has licensed many serological reagents for determination of human

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erythrocyte antigens. While manual methods are still used to perform serologic testing, many hospitals and testing laboratories now utilize automated instruments. Although these instruments vary in design, they all share software controlled electro-mechanical devices to perform such functions as pipetting and plate preparation, incubation, washing, centrifugation and shaking, bar coding for sample and reagent/component tracking, and cameras and software for image capture and interpretation. Automated process controls and error detection mechanisms are often included to reduce or eliminate opportunities for user error and to invalidate suspect results. New technologies such as monoclonal reagents, solid phase technology, and column agglutination have been developed. However, even with the introduction of automation and monoclonal reagents there are several limitations to using serological methods. Screening large numbers of donor units for all potential antigens using serological testing is impractical because of the limited availability of reagents and the different testing parameters for each reagent. The polyclonal source material used to manufacture licensed serological reagents may vary between lots resulting in variability in performance. Monoclonal reagents may produce discrepant results due to differences in reactivity of reagents that are of the same specificity but use different clones or diluents. And lastly, licensed antisera to most rare antigens are often unavailable, requiring laboratories to use laboratory developed tests and research reagents, if available.

## **VI. MARKETING HISTORY**

The initial version of the PreciseType bead chip was first made available in the US in 2005 as a Research Use Only (RUO) product. After some preliminary studies and experiences with the assay and system, a project was initiated in 2007 to modify the panel of SNPs available on the chip in order to detect additional antigens in response to requests from the users of the research use product. This became HEA BeadChip version 1.2, the version that is being submitted as part of this PMA application, currently named PreciseType.

Since the introduction of PreciseType, over 690,000 tests have been processed using the BAS bead chip technology. In May 2010, PreciseType was CE marked in accordance with the European Union IVD Directive. A total of 54 sites have used at least one kit lot since the introduction of the test in 2005, and there are currently 40 domestic and foreign centers using the BAS technology.

## **VII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH**

As summarized in Table 6, the use of the PreciseType DNA microarray assay in transfusion medicine offers several benefits for both donor and patient testing.

**Table 6: Benefits of Molecular Testing**

<b>Donor Testing</b>	<b>Patient Testing</b>
Resolve typing discrepancies and unusual serological findings	Predict the correct red cell phenotype of multiply transfused individuals
Test for antigens when there is no available reagent antisera	Predict the correct red cell phenotype in individuals with autoantibodies or a positive direct antiglobulin test
Identify red blood cell antigen matched blood for chronically transfused patients	Perform extended phenotype to provide red blood cells antigen matched blood for chronically transfused patients
Adaptable to high throughput platforms and multiplex assays	

However, as with all assay methods, there are limitations with the PreciseType kit:

- The genotype does not always correlate with the phenotype due to samples with rare null phenotypes.
- The assay is not designed to detect all rare or new variants of an antigen.
- The kit involves complex technology and requires training and certification of technologists prior to use of the system.

These limitations may cause false positive or false negative red blood cell typing results of donor and patient samples, resulting in adverse effects to the patient as summarized below in Table 7:

**Table 7: Potential Adverse Effects of PreciseType on Patient Health**

<b>Sample Type</b>	<b>RESULT</b>	<b>POTENTIAL OUTCOME</b>	<b>RISK to Health</b>
Donor	False Negative	Patient with a clinically significant antibody may be transfused with antigen positive blood which may cause a hemolytic transfusion reaction.	High
Donor	False Positive	Donor unit would not be chosen for patient transfusion. Would require testing of additional donor units. May cause unnecessary delay in transfusion of patient and increase the cost of testing.	Low
Patient	False Negative	Lab would erroneously conclude that antigen negative blood is required for the patient. May cause unnecessary delay in transfusion of patient and increase the cost of testing.	Low
Patient	False Positive	<ul style="list-style-type: none"> <li>• Patient may be transfused with antigen positive blood which may cause a hemolytic transfusion reaction if patient has a clinically significant antibody.</li> <li>• Patient may make an antibody they did not previously have, complicating future transfusions.</li> </ul>	High



BAS has mitigated these risks by including a list of known limitations of the assay in the Instructions for Use document and requiring that operators participate in the PreciseType HEA Molecular BeadChip Test Training Program prior to performing the assay.

### VIII. ANALYTICAL STUDIES

- a. **Accuracy Study** - The study was performed internally at BAS and included 1768 unique sample results from the three studies described in Table 8:

**Table 8. Dataset Heterogeneity**

Source of Variation	Data Source		
	<i>Results from the Genotype Detection Study</i>	<i>Results from the European Evaluation Study for CE marking.</i>	<i>DNA Samples from the Clinical Trial Comparison Study</i>
<i>Sample number (unique valid sample results)</i>	88	855	825
<i>Sample type</i>	Well-characterized samples from BAS library	Clinical	Well-characterized clinical samples
<i>DNA concentration</i>	10 ng/μL (LOD) (5 samples <10 ng/μL)	Unknown	20 ng/ μL (LOD)
<i>Initial sample results &gt; 0 ICs</i>	Retrospective study. No retesting.	Retrospective study. No retesting.	PreciseType assay retested.
<i>Positive controls</i>	In-house controls used by BAS during manufacturing and testing.	Each site used their own internal controls	Reference panel BeadCheck

Although the combined data are heterogeneous with respect to sample acquisition, testing, and comparator, the use of samples from the three different sources allowed BAS to achieve a sample size of at least 300 for most antigens.

Two different methods were used to characterize the samples; serology and bi-directional sequencing:

- Red blood cell antigens characterized using serology (licensed antisera): Di<sup>a</sup>, Fy<sup>a</sup>, Fy<sup>b</sup>, M, N, S, s, Jk<sup>a</sup>, Jk<sup>b</sup>, Kp<sup>a</sup>, Kp<sup>b</sup>, Lu<sup>b</sup>, C, c, E, e, K, k (U is inferred from S/s typing)

- Red blood cell antigens characterized using bi-directional sequencing (corresponding licensed antisera are not currently available): Co<sup>a</sup>, Co<sup>b</sup>, Di<sup>b</sup>, HbS, Js<sup>a</sup>, Js<sup>b</sup>, Lu<sup>a</sup>, LW<sup>a</sup>, LW<sup>b</sup>, V, VS, Sc1, Sc2, Do<sup>a</sup>, Do<sup>b</sup>, Jo<sup>a</sup>, Hy

**Pre-specified Acceptance criteria:** Agreement of PreciseType results to the established method (serology or sequencing) is >99% at the lower bound of the one-sided 95% confidence interval. Three hundred samples are needed for each antigen (with 100 percent correct calls) in order for that antigen to meet the acceptance criteria.

**Results:** Percent correct calls were calculated for each antigen. The lower bound of the one-sided 95% confidence limits (LCL) ranged from 98.46% to 99.78%. All antigens met the acceptance criteria except Lu<sup>b</sup> (LCL = 98.46%) and V (LCL = 98.92%).

The Lu<sup>b</sup> antigen had 14 false positive calls out of 1414 comparisons; all wrong calls were on comparisons from the European study. A few of the discordant results were possibly due to the inhibitor or suppressor gene In(Lu) which results in little or no antigen expression. This could not be confirmed because the study was retrospective and further testing of the study subjects was not possible. The occurrence of this mutation is 0.03% in the US population. PreciseType is not designed to detect the In(Lu) gene and this limitation is included in the Limitations section of the Instructions For Use document.

The V antigen had four discordant results (two false positive and false negative) out of 843 comparisons to bi-directional sequencing results. Investigation with an alternate primer combination was able to resolve both alleles necessary for the correct V antigen determination via sequencing, resulting in complete sequencing concordance with the original PreciseType test result.

In conclusion, the study demonstrates that PreciseType accurately determines the genotype and predicted phenotype when compared with established comparative methods (bi-directional sequencing and serology).

- Whole Blood Sample Storage** – Results of this study demonstrate that whole blood drawn and stored in the anticoagulant K3 EDTA is stable between 2 °C and 8 °C for 46 days.
  - Purified DNA Stability** –Results of this study demonstrate that DNA samples can be stored for at least six months at -20 °C.
  - Kit Stability** – Kit stability was evaluated at specified time intervals using opened (multiple uses) and unopened kits. The acceptance criterion states that the assay must generate the correct genotype and phenotype calls for all samples used in the study.
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The results showed that the unopened kits are stable for 11 months from the time of manufacture. Opened kits are stable for six months under normal use.

- e. Lot interchangeability** – A study was performed to demonstrate interchangeability between lots of the DNA extraction kits (QIAamp DSP DNA Blood Mini Kit) when used with the HEA 1.2 PreciseType kit. DNA was extracted using various lots of the automated QIAamp method. Acceptable performance of PreciseType was obtained independent of QIAamp lot.
- f. Assay Guard Band Studies** – Twenty-two studies were performed to validate assay parameters outlined in the Instructions for Use (IFU) document. In each study only the component being studied was varied, all other assay components and procedures were held constant as specified in the IFU. All guard band studies were analyzed for low signal, indeterminate calls, failed results, average lower intensity probe performance, and general trends of all average adjusted intensities across all probes. The results of these studies demonstrate that when the assay is run within the parameters determined to yield acceptable results, the test performs consistently and that the defined parameters are within the boundaries of the acceptable performance range.
- g. Limit of Detection (Minimum amount of DNA)** – The study demonstrated that 15 ng/μL is the lowest concentration in which the assay obtains 95% positive data points for each lot.
- h. Interfering Substances:** No effect on assay performance was observed for the following substances:
- Endogenous – The study included varying amounts of triglycerides, bilirubin (unconjugated), hemoglobin, and total protein in whole blood.
  - Exogenous – Fifteen exogenous substances commonly found in blood were tested at their maximum therapeutic concentrations (antibiotics, pain killers, blood thinners, anti-inflammatory drugs, cholesterol lowering drugs, vitamins, beta-blockers, adrenergic blockers, allergy medicine).
  - Microorganisms – Blood samples were inoculated with low and high concentrations of 15 microorganisms commonly found in the blood and on the skin.
  - Anticoagulant – The study included testing 300 whole blood samples collected in the following anticoagulants – K3 EDTA, Sodium Heparin, Sodium Citrate, CPDA-1 and CPDA-1 with Optisol™ Red Cell Preservative.
- i. Cross Hybridization Studies** – Well characterized samples were run in a ---(b)(4)--- PCR reaction to evaluate whether cross hybridization occurs in the PreciseType Assay. Genotype results obtained by ---(b)(4)--- PCR reaction and by PreciseType were compared to the known genotypes of the well-characterized samples. Upon comparison, no unanticipated genotypes were identified by PreciseType indicating that no cross hybridization was detected.
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- j. Reproducibility and Repeatability (R&R) Studies** - The objective of these studies was to demonstrate that PreciseType generates reproducible and repeatable results with a panel of human DNA samples across sites and operators over five days. The studies were performed with both the eight chip slide and the 96 chip plate.

**Study Design, Plate Format** – A total of eight operators across four sites participated in the study. Training, including proficiency testing, was completed prior to study initiation. The panel consisted of eleven previously characterized (bi-directional sequencing) DNA samples extracted from immortalized cell lines derived from human whole blood and representing all positive phenotypes in PreciseType. BAS provided the blinded samples in the 96-well PCR plates.

**Study Design, Slide Format** - A total of six operators across three sites participated in the study. Training including proficiency testing was completed prior to study initiation. The training panel consisted of eleven previously characterized (bi-directional sequencing) DNA samples extracted from immortalized cell lines derived from human whole blood representing all positive phenotypes in PreciseType. BAS provided the blinded samples in 96-well PCR plates.

An assay run was repeated if it was determined to be invalid, (i.e., operator error, apparent equipment failure, or a negative or positive control not valid).

Samples with invalid results (high background, low signal, indeterminate call, high coefficient of variation) were categorized as no type determined (NTD) and not included in calculations (Slide – 0.3% invalid sample rate, Plate – 0.8% invalid sample rate).

**Repeatability results:** The comparison of interest is antigen replicates for PreciseType. For both the plate and slide formats, all antigens had either all positive or all negative results for each sample. Therefore, the results showed 100% agreement and the studies showed 100% repeatability.

**Reproducibility results:** For both the plate and slide format all results combined across operators, sites, and days agreed with each other for each antigen in each sample. Therefore, these studies showed 100% reproducibility.

### **Lot-to-Lot Reproducibility Study**

**Study Design** - This study was conducted at a single internal site (BioArray Solutions) to evaluate the reproducibility of three lots of PreciseType using a fully characterized panel (n=22) of extracted human genomic DNA samples. These 22 samples were selected to represent the broadest ranges of alleles possible that are contained in PreciseType. The assay on each sample was performed using

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PreciseType kits from three different lots to demonstrate the lot-to-lot reproducibility. The same assays were repeated by the same operator on five separate days to demonstrate the repeatability. To eliminate bias, all samples were blinded such that the composition of the Reproducibility Panel samples was unknown to the operator.

**Lot-to-Lot Results** – The comparison of interest is antigen replicates for PreciseType. All antigens had either all positive or all negative results for each sample. Therefore, the results showed 100% agreement and the study showed 100% lot-to-lot repeatability and reproducibility.

## IX. CLINICAL STUDIES

### Comparison of PreciseType to established methods:

**Study Design** - The method comparison study was a multi-site, prospective study that evaluated the performance of PreciseType in comparison to serological testing and bi-directional sequencing in DNA samples obtained from donor or recipient whole blood specimens. The study was a paired evaluator study design in which specimens are either evaluated by serological reagents and PreciseType or; for rare alleles and where there is no FDA licensed serological reagent; evaluated by bi-directional sequencing and PreciseType.

- Blood antigens using serology as the comparison method: Di<sup>a</sup>, Fy<sup>a</sup>, Fy<sup>b</sup>, M, N, S, s, Jk<sup>a</sup>, Jk<sup>b</sup>, Kp<sup>a</sup>, Kp<sup>b</sup>, Lu<sup>b</sup>, C, c, E, e, K, k (U is inferred from S/s typing)
- Blood antigens using sequencing as the comparison method (corresponding licensed antisera are not currently available): Co<sup>a</sup>, Co<sup>b</sup>, Di<sup>b</sup>, HbS, Js<sup>a</sup>, Js<sup>b</sup>, Lu<sup>a</sup>, LW<sup>a</sup>, LW<sup>b</sup>, V, VS, Sc1, Sc2, Do<sup>a</sup>, Do<sup>b</sup>, Jo<sup>a</sup>, Hy

Four qualified immunohematology/molecular laboratory sites participated in this study. The study sites were selected for the diversity of their locations and donor populations. See Table 9 below for a summary of the donor demographics, for all sites.

**Table 9: Study Demographics for all Testing Sites**

<b>Age</b>	16 – 85 years	
<b>Race/Ethnicity</b>	American Indian or Alaska Native	3.1% (n = 41)
	Asian	21.8% (n = 288)
	Black or African American	48.3% (n = 637)
	Hispanic or Latino	9.3% (n = 123)

	Mixed Race	0.3% (n = 4)
	Native Hawaiian or Other Pacific Islander	1.5% (n = 20)
	Not provided	0.8% (n = 11)
	White	14.5% (n = 191)

All site personnel were trained by the Technical Site Representative and the Study Monitor on the study procedures and protocol.

Four lots of PreciseType were used in the study. A total of 1757 whole blood samples (EDTA Vacutainer® collection tubes) were collected from blood donors or patients at each of the study sites. DNA extraction was performed on all samples with the QIAGEN QIAamp DNA Blood Mini Kit. Samples used in the study were leftover specimens from each laboratory's routine clinical analysis that would normally be discarded. All samples were de-identified and assigned unique study identification numbers.

The FDA licensed serological reagents were provided by each site and included reagents typically used for routine clinical analysis. Bi-directional DNA sequencing was performed by a contract testing laboratory. At BAS an independent analysis of the sequencing data and the translation to phenotypes were performed by three trained individuals, blinded to the results.

If there was disagreement between PreciseType and serology results, or between PreciseType and DNA sequencing results, DNA sequencing was used as the referee method to establish the correct genotype of the antigen in question.

**Acceptance criteria:** Overall agreement or concordance of PreciseType results to the comparison method (serology or sequencing) test results are to be >99% at the lower bound of the one-sided 95% confidence interval for each antigen, using data from the initial assay runs.

**Statistical Methods:** Agreement (positive percent agreement [PPA], negative percent agreement [NPA] and overall agreement [OPA]) or concordance (sensitivity, specificity, and overall concordance) was calculated for each individual antigen. The lower bound of the one-sided 95% confidence interval was calculated using the Clopper-Pearson Exact Method. Agreement or concordance was calculated using initial test results prior to the analysis of disagreement or discordance.

**Comparability Results:**

• **Precise Type Versus Serology Results**

After discrepancy resolution, the PreciseType demonstrated comparable performance to serology for 18 of the 19 antigens listed below:

c, C, e, E, K, k, Kp<sup>a</sup>, Kp<sup>b</sup>, Jk<sup>a</sup>, Jk<sup>b</sup>, Fy<sup>a</sup>, Fy<sup>b</sup>, M, N, S, s, Lu<sup>b</sup>, Di<sup>a</sup>, and U.

The final lower limit of the one-sided 95% confidence interval for Jk<sup>b</sup> was 98.95% agreement. Seven of the 1248 samples were identified as *Jknull*. PreciseType does not cover all molecular variants that affect blood group antigen expression and phenotypes and may generate false-positive results for Jk<sup>b</sup>. This limitation is listed in the IFU.

• **Precise Type versus Sequencing - Results:**

High rates of concordance were observed (lower limit of the one-sided 95% confidence intervals were >99%) between PreciseType and DNA sequencing for the following antigens (no corresponding licensed antisera are currently available):

Js<sup>a</sup>, Js<sup>b</sup>, Lu<sup>a</sup>, Di<sup>b</sup>, Co<sup>a</sup>, Co<sup>b</sup>, Do<sup>a</sup>, Do<sup>b</sup>, Jo<sup>a</sup>, Hy, LW<sup>a</sup>, LW<sup>b</sup>, Sc1, Sc2, HbS, VS, and V

**X. INSPECTIONS**

**A. Manufacturing Facility**

FDA conducted an inspection at the following facility:

BioArray Solutions  
35 Technology Drive, Suite 100  
Warren NJ 07059  
Establishment Inspection Dates: November 4 – 15, 2013  
FEI#: 3005967741

This inspection was classified as Voluntary Action Indicated (VAI) with the issuance of an FDA Form 483. Sixteen observations were identified relating to the following areas:

- Receiving, reviewing and evaluating complaints
- Documentation of corrective and preventive actions
- Procedures for controlling nonconforming product
- Documentation of process validation activities
- Reconciliation of final kits and carriers/slides
- Design and process changes
- Design transfer
- Supplier agreements and evaluations
- Shipping

- Control of product in storage areas
- Complaint files
- Procedures for internal quality audits

BAS responded to the inspectional observations on the following dates:

- December 6, 2013
- January 8, 2014
- February 6, 2014
- March 7, 2014

BAS has committed to correcting all deficiencies. FDA has determined the BAS responses are adequate.

#### **B. BioResearch Monitoring (BIMO) Inspection**

CBER Bioresearch Monitoring (BIMO) Branch issued high-priority inspection assignments at three testing sites in the United States. The BIMO inspection at the New York Blood Center revealed problems that impacted the data submitted in the pre-marketing application (PMA): serology testing for 446 samples was completed by an undisclosed entity using un-licensed antisera for the following antigens: Jk<sup>a</sup>, Jkb, S, s, Fy<sup>a</sup>, and Fyb. The testing for those antigens was subsequently repeated for 437 samples using licensed antisera.

Inspections at the other two sites yielded no information that would impact the data.

### **XI. DEVICE PANEL MEETING RECOMMENDATIONS**

On March 18, 2014, the Blood Products Advisory Committee (BPAC) met as a device panel to assess the safety and effectiveness of PreciseType. BPAC heard presentations by BAS on the design and performance of the test system and by FDA on its analysis of the performance studies.

FDA posed one question to BPAC and asked for comments on three areas.

1. Please comment whether the data support accuracy of the Immucor PreciseType™ HEA Molecular BeadChip Test to determine the blood group antigen genotype and predicted phenotype.

The consensus of the Committee was that the data presented were robust and supported the accuracy of the test to determine the blood group antigen genotype and predicted phenotype.



2. Please comment on the applicant's training program to ensure end users are properly trained to obtain reliable results.

The Committee responded that the training program is adequate, but noted that it will be important for the users to maintain competencies and to participate in retraining when not using the test frequently.

3. Please comment on the applicant's proposal to address new molecular variants.

The Committee members found the proposal to address new molecular variants to be adequate.

4. Do the available data provide reasonable assurance of the safety and effectiveness of the Immucor PreciseType™ HEA Molecular BeadChip Test for its intended use?

The Committee agreed unanimously (12 yes votes, 0 no votes).

## **XII. CBER DECISION**

The available data provide reasonable assurance that PreciseType is safe and effective for its intended use and support approval of PreciseType.