CPAL

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RBC Antigen Genotyping
- New Test -

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Ordering Information and Suggested Codes:

CPAL Test Name	Lab Nexus Panel Code	CPT Code
HEA Blood Genotyping	HEA	81403
RhCE Blood Genotyping	RhCE	81403
RhD Blood Genotyping	RhD	81403

Effective Date: Testing offered as of February 1, 2019.

Performed: One day per week on dayshift. Samples will be extracted and held as they are received and tested on Thursdays. As volumes increase, the testing schedule will be adjusted accordingly.

Testing Method: DNA Extraction; Elongation-mediated Multiplexed Analysis of Polymorphisms (eMAP™).

Specimen Requirements:
- Whole blood collected in tubes containing EDTA as the anticoagulant.
- Minimum volume accepted: 1 mL.
- Whole blood is stable at 2°C to 8°C for up to one month. Fresher samples are preferable due to better DNA yield.

Background:
Human erythrocyte blood group antigens (HEA) are located on the membrane of the red blood cell. These surface markers are polymorphic protein and/or carbohydrate structures that are attached to lipid or protein. The PreciseType® HEA BeadChip Kit contains twenty-four polymorphisms associated with thirty-five Human Erythrocyte Antigens and one with hemoglobinopathies. The PreciseType® HEA Molecular BeadChip Test uses the proprietary Elongation-mediated Multiplexed Analysis of Polymorphisms (eMAP®) technology to identify the presence or absence of the selected alleles associated with a given phenotype.

The BioArray Solutions RHD BeadChip Kit and RHCE BeadChip Kit also uses the Elongation-mediated Multiplexed Analysis of Polymorphisms (eMAP®) technology to identify the presence or absence of the selected alleles associated with a given phenotype.
DNA extraction of whole blood and multiplex PCR analysis are used to isolate and amplify DNA specimen. Post-PCR processing is then performed using Clean-up Reagent and Lambda Exonuclease to produce single-strand target DNAs or amplicons. The single-stranded DNAs or amplicons are incubated on the BeadChip array, allowing the annealing with the corresponding blood-group-specific probes. The BioArray Array

Imaging System™ is used to capture the fluorescent signal and processes the data generated. The BioArray Solutions Information System (BASIS®) analyzes the data and generates assay results.

Contents of Panels:
For a list of genetic markers contained in each panel and the expected phenotypes, please see the tables in the Appendix of this document.

Validation:
Included in the validation were method correlations of the HEA, RhD, and RhCE assays using vendor-provided DNA panels and whole blood samples obtained from members and reference laboratories used by members. The results were within acceptable parameters and are available onsite for review upon request.

Limitations:
1. Any results that are generated must be interpreted in conjunction with other clinical or laboratory findings.
2. BioArray Solutions RHD and RHCE BeadChip kits are for Research Use Only. These tests are not intended for clinical diagnosis or as the sole means for patient management decisions.
3. False positive and/or invalid HEA results may be generated in rare cases where a sample contains examples of molecular events that affect the blood-group antigen expression and phenotypes and the nucleotide changes associated with these events are not explicitly monitored by the assay. Examples include 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. 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 result.
4. False negative and/or HEA invalid results may be generated when unanticipated rare mutation(s) affecting the primer or probe binding cause allele and/or amplicon dropout.
5. The RHD kit is unable to differentiate between homozygous and hemizygous forms of alleles.
6. The RHD kit is unable to detect hybrid alleles in a heterozygous combination with other alleles, when the hybrid allele does not have any characteristic markers outside the hybrid region.
7. The RHCE assay is unable to detect RHCE-RHD hybrids, except for CeRN. The CeRN allele can only be detected in the homozygous state.
8. Certain alleles cannot be detected using the RHCE assay because of the absence of relevant markers in the RHCE BeadChip panel. These alleles may be mistyped as alleles that are covered by the RHCE assay.

References:

1. Immucor BioArray PreciseType HEA Molecular BeadChip Test doc 190-20210-EN Rev. C Oct 2016.
2. Immucor BioArray PreciseType RHCE Molecular BeadChip Test doc 190-00307-EN Rev. G Oct 2015.
3. Immucor BioArray PreciseType RHD Molecular BeadChip Test doc 190-00302-EN Rev. E Oct 2015.
4. Immucor ETC AIS Exposure Test P/N 190-20474 Rev. A Date of Revision: July 2016
5. Immucor PreciseType HEA Test User Training Guide, doc 190-20333 Rev B, 2014.

APPENDIX

Genetic Markers for Red Blood Cell Antigens in the PreciseType HEA Test:

Blood Group System	Analyte	Polymorphism	ISBT Phenotype	*ISBT Genotype*
Rh	c/C	307 C>T	RH4, RH2	*RHCE*4, RHCE*2*
		109 Ins		
	e/E	676 G>C	RH5, RH3	*RHCE*5, RHCE*3*
	VS	733 C>G, 1006 G>T	RH20	*RHCE*01.20.01, RHCE01.20.02, RHCE*01.20.04, RHCE*01.20.05*
	V		RH10	
Kell	K/k	698 T>C	KEL1, KEL2	*KEL*01, KEL*02*
	Js^a/Js^b	1910 C>T	KEL6, KEL7	*KEL*06, KEL*07*
	Kp^a/Kp^b	961 T>C	KEL3, KEL4	*KEL*03, KEL*04*
Duffy	Fy^a/Fy^b	125 G>A	FY1, FY2	*FY*01, FY*02*
	GATA (Silencing *FY*)	-67 T>C**	FY-2	*FY*02N.01*
	$Fy^x[Fy(b+^w)]$	265 C>T	FY2W	*FY*02M*
Kidd	Jk^a/Jk^b	838 G>A	JK1, JK2	*JK*01, JK*02*
MNS	M/N	59 C>T	MNS1, MNS2	*GYPA*01, GYPA*02*
	S/s	143 T>C	MNS3, MNS4	*GYPB*03, GYPB*04*
	Silencing S (Uvar, Uneg)	230C>T	MNS-3, 5W, MNS-3,-4,-5	*GYPB*03N.01 or GYPB*03N.02*
		In5 g>t		*GYPB*03N.03 or GYPB*03N.04*
Lutheran	Lu^a/Lu^b	230 A>G	LU1, LU2	*LU*01, LU*02*
Dombrock	Do^a/Do^b	793 A>G	DO1, DO2	*DO*01, DO*02*
	Hy+/Hy-	323 G>T	DO4	*DO*04*
	Jo(a+)/Jo(a-)	350 C>T	DO5	*DO*05*
Landsteiner-Wiener	LW^a/LW^b	308 A>G	LW5, LW7	*LW*05, LW*07*
Diego	Di^b/Di^a	2561 C>T	DI2, DI1	*DI*02, DI*01*
Colton	Co^a/Co^b	134 C>T	CO1, CO2	*CO*01, CO*02*
Scianna	Sc1/Sc2	169 G>A	SC1, SC2	*SC*01, SC*02*

** The GATA mutation listed here has been previously reported at -33 and -46 (ISBT Working Party)[9].

Genetic markers in the RHD BeadChip Kit:

Amino Acid	Nucleotide Polymorphism
S3C	8 C>G
W16C	48 G>C
W16X	48 G>A
L62F	186 G>T
R70Q	209 G>A
R114W	340 C>T
A137V	410 C>T
A149D	446 C>A
N152T	455 A>C
IVS3+1G/A	In3+1G>A
psi D	In3 -19 37 bp duplication
M170T	509 T>C
I172F	514 A>T
T201R	602 C>G
F223V	667 T>G
A226P	676 G>C
S230I	689 G>T
E233Q	697 G>C

Amino Acid	Nucleotide Polymorphism
E233K	697 G>A
V238M	712 G>A
V245L	733 G>C
G263R	787 G>A
Y269X	807 T>G
V270G	809 T>G
V279M	835 G >A
G282D	845 G > A
T283I	848 C>T
M295I	885 G>T
I342T	1025 T>C
D350H	1048 G > C
G353W	1057 G>T
G355S	1063 G>A
G385A	1154 G>C
E398V	1193 A>T
1227G/A	1227 G>A

Phenotypical variants detected by the RHD BeadChip kit:

BeadChip names	ISBT Names [5]
possible D[9]	RHD*01
1227A (Del)	RHD*01EL.01
RHD Deletion	RHD*01N.01
RHD-CE(3-9)-D	RHD*01N.04
RHD-CE(3-7)-D	RHD*01N.06
RHD-CE(4-7)-D	RHD*01N.07
48A (W16X)	RHD*01N.08
807G (Y269X)	RHD*01N.18
weak D type 1	RHD*01W.1
weak D type 1.1	RHD*01W.1.1
weak D type 14 or 40 or 51	RHD*01W.14 or RHD*01W.40 or RHD*01W.51
weak D type 17	RHD*01W.17
weak D type 2	RHD*01W.2
weak D type 47	RHD*01W.47
weak D type 29	RHD*01W.29
weak D type 3	RHD*01W.3
weak D type 34	RHD*01W.34
weak D type 41	RHD*01W.41
weak D type 5	RHD*01W.5
DIIIa	RHD*03.01
DIIIb	RHD*03.02
DIIIc	RHD*03.03
DIII type 4	RHD*03.04
DIII type 6 or DIII type 7	RHD*03.06 or RHD*03.07
DIII type 7	RHD*03.07
DIVa	RHD*04.01
DIVa type 2	RHD*04.02
DIV type 3	RHD*04.03
DIV type 4	RHD*04.04
DIV type 5 or DIVb	RHD*04.05 or RHD*04.06
DIVb	RHD*04.06
RHD psi	RHD*04N.01
DV type 2 or DBS1	RHD*05.02 or RHD*13.01
DV type 2 or DBS1 or DV type 7	RHD*05.02 or RHD*13.01 or RHD*05.07

BeadChip names	ISBT Names [5]
DBS0	RHD*05.03
DV type 4	RHD*05.04
DV type 6	RHD*05.06
DV type 8	RHD*05.08
DV type 9	RHD*05.09
DVI	RHD*06
DAR	RHD*09.01
DAR-E	RHD*09.02
weak D type 4.0 or 4.3	RHD*09.03 or RHD*09.05
weak D type 4.1	RHD*09.04
DAU1	RHD*10.01
DAU2	RHD*10.02
DAU3	RHD*10.03
DAU4 or DV type 5	RHD*10.04 or RHD*05.05
DAU5 or DV type 1 or DBS2	RHD*10.05 or RHD*05.01 or RHD*13.02
weak D type 11	RHD*11
DOL or DOL2	RHD*12.01 or RHD*12.02
DOL3	RHD*12.03
DBT1	RHD*14.01
DBT2	RHD*14.02
weak D type 15	RHD*15
DCS1 or DFV	RHD*16.01 or RHD*30
DCS2	RHD*16.02
DFR or DFR3	RHD*17.01 or RHD*17.03
DFR2	RHD*17.02
DFR4	RHD*17.04
DHMi	RHD*19
IVS3+1G>A (Del)	RHD*208
DNB	RHD*25
DUC2	RHD*37
DIIIa-CE(4-7)-D[59]	N/A [6]
RHCE(1-3)-D(4-10)	N/A [7]
ceHAR	RHCE*01.22
RHD Deletion (possible rG)	RHD*01N.01 (possible RHCE*02.03)

Table 3c: D Negative Hybrid Alleles:

Hybrids with RHD negative phenotypes
RHCE(1-3)-D(4-10)
RHD-CE(3-7)-D
RHD-CE(3-9)-D
RHD-CE(4-7)-D
DIIIa-CE(4-7)-D

Genetic markers in the RHCE BeadChip Kit:

Amino Acid	Nucleotide Polymorphism
W16C	48 G>C
A36T	106 G>C
Q41R	122 A>G
P103S	307 C>T
*109Ins	109 bp intron 2 insert
R114W	340 C>T
L115R	344 T>G
S122L	365 C>T
T152N	455 C>A
R154T	461 G>C
M167K	500 T>A
G180R	538 G>C
R201T	602 G>C

Amino Acid	Nucleotide Polymorphism
V223F	667 G>T
A226P	676 G>C
Q233E	697 C>G
M238V	712 A>G
L245V	733 C>G
V250M	748 G>A
dT744dC	744 T>C
A273V	818 C>T
I306V	916 A>G
G336C	1006 G>T
T342I	1025 C>T
Rh r^S	Cde^S 5'UTR

* 109Ins detects the presence or absence of 'C' in samples. It is not a marker for 'c'

Phenotypical variants detected by the RHCE BeadChip kit:

Conventional Name (Case-Sensitive)	ISBT Name [5]
WT(ce)	RHCE*01
WT(Ce)	RHCE*02
WT(cE)	RHCE*03
WT(CE)	RHCE*04
ce(48C)	RHCE*01.01
ceTI	RHCE*01.02
ce(1025T)	RHCE*01.03
ceAR	RHCE*01.04
ceEK	RHCE*01.05
ceMO	RHCE*01.07
ceBI or ceSM	RHCE*01.08 or RHCE*01.09
ceSL	RHCE*01.10
ceRT	RHCE*01.11
ceRA	RHCE*01.12
ce(733G)	RHCE*01.20.01
ce(48C,733G)	RHCE*01.20.02
ce(48C,733G,1006T)§	RHCE*01.20.03
ce(48C,733G,1025T)	RHCE*01.20.04
ce(733G,1006T)§	RHCE*01.20.05
ceCF	RHCE*01.20.06
ce(697G,733G)	
ceJAL	RHCE*01.20.07
ce(48C,340T,733G)	
ce(48C,733G,748A)	RHCE*01.20.08

Conventional Name (Case-Sensitive)	ISBT Name [5]
ce(733G,748A)	
ceHAR	RHCE*01.22
CeMA	RHCE*02.01
CeFV	RHCE*02.02
Ce(365T)	RHCE*02.03
CeVA	RHCE*02.04
CeCW	RHCE*02.08.01
ce(48C,122G)	
CeCX	RHCE*02.09
ce(48C,106A)	
CeRN	RHCE*02.10.01
Ce(344G)	RHCE*02.12
cEEW	RHCE*03.01
cEFM	RHCE*03.03
cE(602C)	RHCE*03.04
cEKH	RHCE*03.05
cE(344C)	RHCE*03.07
(48C,712G,733G) [11]	
(340T,344G) [10]	
ce(48C,697G,733G,1006T)§ [9]	
ce(48C,697G,712G,733G,916T)[12]	
ce(48C,733G,744C) [13]	
Ce(667T)	
cE(365T)	

Notes: The presence of alleles marked with "§" may suggest the presence of (C)ces or r's haplotype. For confirmation, test the sample for presence of DIIIa-CE(4-7)-D using the RHD BeadChip kit. The above tables are not all inclusive of all possible RHCE genetic variations. See the Limitations section for RHCE variations not covered by this test.