RH is the most complex blood group system, with over 40 antigens encoded by two highly homologous genes: RHCE and RHD. Because of RH's high immunogenicity, this system is critical in blood banking and is associated with transfusion reactions and hemolytic disease of the newborn.

**Background**

Applications of RH variant typing by DNA analysis may include:
- identifying clinically significant RH polymorphisms undetectable by current serological techniques
- investigating anomalous or ambiguous serological typings (e.g. unexpected RH antibodies, weak reactions, variations in reactivity across reagents)
- conducting detailed RH variant analysis in pregnant women (e.g. partial D, weak D)
- selectively testing RH variant analysis in pregnant women (e.g. partial D, weak D)

**Method:**

**eMAP™ protocol**

DNA isolation

- DNA is extracted and isolated

Amplification

- DNA segments of interest are amplified using multiplex PCR

Clean up & Digestion

- PCR product is processed to remove residual primers and dNTPs and single stranded DNA is generated

Annealing & Elongation

- Amplified DNA anneals with the oligonucleotide probe on its target bead; perfectly matched probes elongate

Detection

- Bead fluorescence profile is analyzed to assess bead elongation pattern

Interpretation

- Bead fluorescence profile is translated into genotype and phenotype interpretations via BASIS (BioArray Solutions Information system)

**RHD Validation Summary:**

Validation of the RHD BeadChip assay included an evaluation of accuracy, precision, sensitivity, specificity and interfering substances. Accuracy of the RHD assay demonstrated 99.9% concordance between the Immucor DX Laboratory, a reference laboratory, and BioArray Solutions Research and Development Laboratory. The RHD Beadchip assay demonstrated 92.6% sensitivity and 96.1% specificity when compared with established reference methods. Interference testing of common endogenous interfering substances as well as exogenous substances and microorganisms demonstrated no impact on the performance of the RHD BeadChip assay.

**Accuracy** 99.9%

**Sensitivity** 92.6%

**Specificity** 96.1%

**Interfering Substances** No Impact

**RHCE Assay Variant Coverage**

- Detects C, c, E, e, VS, and V antigens along with 44+ RHCE variants including ceAR, ceBI or ceSM, ceCF, CeCW, CeCX, ceEK, ceEW, cEFM, CeIV, ceJAR, ceJAL, cEKH, CeMA, ceMO, ceRA, CeRN, ceRT, ceSL, ceTI, CeVA, r’s, ce(1025T), cE(344C), Ce(344G), cE(365T), Ce(365T), cE(365T), cE(602C), Ce(667T), ce(697G,733G), ce(733G), ce(733G,1006T), ce(733G,748A), ce(48C), ce(48C,106A), ce(48C,122G), ce(48C,340T,733G)

**RHD Assay Variant Coverage**

- Weak D type: 1, 1.1, 2, 3, 5, 14, 17, 29, 34, 40, 41, 47, 51
- D negative: RHD deletion, RHD-Ψ, RHD-CE(3-9)-D, RHD-CE(3-7)-D, 48A (W16X), 807G (Y269X), DIIIa-CE(4-7)-D, RHCE(1-3)-D(4-10)
- Del: 1227A, IVS3+1G>A
- Partial D: DBS0,1,2; DAR, DAR-E, DAI 1,2,3,4,5; DOL 1,2,3; DBT1,2; DIIIa,b,c; DIII type 4,6,7; DIVa,b; DIVa-2, DIV type 3,4,5; DV type 1,2,4,5,6,7,8,9; DVI, DCS1,2; DFR1,2,3,4; DHMi, DNB, DUC2, ceHAR, DFV, Weak D type 4.0, 4.1, 4.3, 11, 15
RHCE Validation Summary:

Validation of the RHCE BeadChip assay included an evaluation of accuracy, precision, sensitivity, specificity and interfering substances. Accuracy of the RHCE assay demonstrated 100% concordance between the Immucor DX Laboratory, a reference laboratory, and BioArray Solutions Research and Development Laboratory. The RHCE BeadChip assay demonstrated 99.0% sensitivity and 99.0% specificity when compared with established reference methods. Interference testing of common endogenous interfering substances as well as exogenous substances and microorganisms demonstrated no impact on the performance of the RHCE BeadChip assay.

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<tr>
<th>CPT Code</th>
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<td>81403</td>
<td>Molecular pathology procedure</td>
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Complementing Your Organization’s Capability

- Laboratories without onsite molecular immunohematology capabilities can now effectively reflex testing to the Immucor DX Reference Laboratory.
- Leverage the capabilities of molecular diagnostics without the capital outlay and maintenance of an onsite molecular laboratory.

The process is quick and simple:

1. Collect whole blood or DNA sample
2. Package in Immucor DX lab pak
3. Submit test requisition form and sample for testing
4. Receive results in <1 week

ImmucorDX has achieved both CLIA certification and CAP accreditation!

CLIA Number: 23D1054909
CAP Number: 7195469

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