National Molecular Laboratory
Penn-Jersey Region
American Red Cross
700 Spring Garden Street
Philadelphia, PA 19123-3594
(215) 451-4917
1-800-RED CROSS

Report Date: 07/02/20

INSTITUTION: Provider

SAMPLE: (ML20-1720) Date received:
ID# ; DOB: Date colle

SAMPLE TYPE: Whole Blood

HISTORY: Caucasian male, low hemoglobin. History of transfusion. RBCs type D+. A1 antigen negative.

TESTING REQUESTED: ABO Common allele genotyping and ABO variant testing of warranted.

TESTING PERFORMED: Inno-train RBC-ABO Basic SSP-PCR.
NOTE: This testing does not rule out the presence of ABO subgroups.

Alleles: *ABO*O1* / *ABO*A2*

Predicted Phenotype: Group A_2

COMMENT: The patient expresses the A_2 subgroup. Anti-A1 reacts weakly in 1-8% of group A_2 and 22-35% of group A_2B individuals [Meny GM. *Immunohematology* 2017 33(2):76-81]. ABO variant testing is not warranted.

Mitchell Lindquist, MS
Lead Technologist

Margaret A. Keller, PhD
Sr. Director, National Molecular Laboratory

Please Give Blood.

CLIA #: 39D0194473 **CLIA Laboratory Director: David Moolten, M.D.**

Test Methods
DNA was extracted from whole blood on a Qiagen Qiacube, or manually using Qiagen DNA Extraction kits following manufacturer's instructions. Purified DNA was used as template in SSP-PCR amplification reactions, using the Inno-train RBC-FluoGene RBC-ABO Basic test kit. The RBC-ABO Basic test kit interrogates *ABO* c.261G/delG, c.802G/A, c.803G/C, c.1061C/delC, markers associated with *ABO*01, ABO*02, ABO*B, ABO*A2* as well as *ABO*A* alleles other than *ABO*A2*.

Test Limitations
This testing does not detect polymorphisms other than these assayed, including genetic variants that may result in a null or altered phenotype. The testing cannot definitively assign *ABO*A1* but can rule out *ABO*A2*. The positivity of all "non" reactions with no or maximum one positive specific reaction most likely represents an A1 allele, and is interpreted as A, but rare alleles cannot be excluded. The testing does not detect nucleotides associated with ABO subtypes. The c.261G marker may not be detected in samples carrying *ABO*AW.02*. The c.803C marker may not be detected in samples carrying *ABO*BW.32*. The c.803G marker may not be detected in samples carrying *ABO*cisAB.04, ABO*cisAB.05, ABO*BA.06, ABO*O.15*. The c.803G marker will not be detected in samples carrying *ABO*AW.08, ABO*AW.42, ABO*O.01.57, ABO*O.02.01*, *ABO*O.02.02, ABO*O.02.03, ABO*O.02.04.*

Since these methods involve hybridization and PCR amplification with nucleotide primers, other polymorphisms in the primer or probe binding region can affect the testing, and ultimately, the predicted phenotypes. An individual's phenotype may be affected by non-genetic factors. Findings from this testing should be confirmed by another method. In addition, the genotype obtained from DNA isolated from leucocytes and other hematopoietic cells may differ from that of other tissues in persons with a history of transplantation. The presence of more than two haplotypes (chimerism) can lead to incorrect results.

Disclaimers
The testing performed here is for research use only. These tests were validated by The National Molecular Laboratory. The tests have not been cleared or approved by the Food and Drug Administration (FDA) or other regulating bodies and are, therefore, are not FDA-licensed tests. The National Molecular Laboratory participates in proficiency exchange programs for this testing. These results are intended for predicting blood group antigens in patients and donors, and are not intended for clinical diagnosis or as the sole means for patient management decisions.

Abbreviations
PCR, polymerase chain reaction; SSP-PCR, sequence-specific primer PCR; gDNA seq, genomic DNA sequence analysis; bp, base pair;

References

Blood Group Antigen FactsBook, 3rd ed. ME Reid and C Lomas-Francis 2012
http://www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology/blood-group-terminology/blood-group-allele-terminology/
Meny GM. *Immunohematology* 33(2) 2017.

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Report Date: 08/27/19

INSTITUTION: [redacted]

Physician: [redacted]

SAMPLE: [redacted] (ML19-2172)
Patient ID#: [redacted]; DOB: [redacted]

Date received: 08/23/19
Date collected: 08/21/19

SAMPLE TYPE: Whole blood

HISTORY: Caucasian female, suspected neonatal autoimmune thrombocytopenia (NAIT).

TESTING REQUESTED: HPA Genotyping Panel

TESTING PERFORMED: HPA-FluoGene (includes HPA-1,-2,-3,-4,-5,-6,-9,-15)

HPA	a	b
HPA-1	0	+
HPA-2	+	0
HPA-3	+	0
HPA-4	+	0
HPA-5	+	0
HPA-6	+	0
HPA-9	+	0
HPA-15	+	+

[redacted]
Supervisor, National Molecular Laboratory

[redacted]
Manager, National Molecular Laboratory

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Test Methods
DNA was extracted from whole blood on a Qiagen Biorobot or from buffy coat by Qiacube, or manually using Qiagen DSP DNA Blood Mini kits following manufacturer's instructions. HPA Fluogene (Innotrain DIagnostik) uses SSP-PCR to interrogate the biallelelic SNPs associated with HPA1,-2,-3,-4,-5,-6,-9,-15.

Test Limitations
This testing does not detect polymorphisms other than these assayed, including genetic variants that may result in a null phenotype. Since these methods involve PCR amplification with nucleotide primers, other polymorphisms in the primer binding region can affect the testing, and ultimately, the predicted phenotypes. An individual's phenotype may be affected by non-genetic factors. Findings from this testing should be confirmed by another method when possible. In addition, the genotype obtained from DNA isolated from leucocytes and other hematopoietic cells may differ from that of other tissues in persons with a history of transplantation.

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Abbreviations
AS-PCR; allele-specific PCR, also known as sequence-specific primer (SSP)-PCR

References
1. Metcalfe, P and AH Waters *Br. J. Haem*. 1993 85:227-229.
2. http://www.ebi.ac.uk/ipd/hpa/table2.html

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Report prepared by: RG 08/27/19
Fluogene Run Date: 08/27/19

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Report Date: 07/14/20

INSTITUTION: [redacted] Provider [redacted]

SAMPLE: [redacted] (ML20-1834)
ID# [redacted]; DOB: [redacted]

Date received: 07/08/20
Date collected: 07/06/20

SAMPLE TYPE: Whole Blood

HISTORY: Caucasian female, weak D positive.

TESTING REQUESTED: Genotype for *RHD* variants

TESTING PERFORMED			RESULT
***RHD* Variants**	**Method**	**Analyte: Nucleotide (Amino Acid)**	**Nucleotide(s) Detected**
w*RHD* BEADCHIP™	*RHD* Array*	1154G>C (G385A)	**C**

*Only nucleotides which differ from consensus sequence are listed.

Probable *RHD* Genotype: *RHD*weak D type 2* (hemizygous or homozygous)

Predicted phenotype: Weak D+

COMMENTS: Weak D type 2 RBCs have low D antigen density (Rhesus index 489) and are associated with RhD typing discrepancies.

Individuals with weak D type 2 are not considered at risk of alloimmunization by RhD (Pham BN *et al. Transfusion*. 2011; 51(12):2679-85; Sandler S G *et al. Transfusion* 55(3):680-689).

It is generally accepted that females of child-bearing potential with weak D type 2 can be considered D positive for transfusion and are not candidates for Rh immune globulin.

[redacted]
Lead Technologist

[redacted]
Sr. Director, National Molecular Laboratory

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CLIA #: 39D0194473 **CLIA Laboratory Director: David Moolten, M.D.**

Test Methods
DNA was extracted from whole blood on a Qiagen Qiacube, or manually using Qiagen DNA Extraction kits following manufacturer's instructions. Purified DNA was used as template in PCR amplification reactions, some of which were allele-specific reactions to identify single germline variants. Others were PCR-restriction fragment length polymorphism (RFLP) reactions where the PCR product was incubated with a restriction enzyme and the resulting products resolved using agarose gel electrophoresis. Banding patterns are used to interpret the genotype. Some samples are subjected to DNA sequence analysis of one or more exons. In some cases, RNA is isolated from the red cell fraction of the whole blood and used to synthesize cDNA. The cDNA is used as template for gene-specific PCR amplification. In some instances, PCR products are cloned into plasmids prior to DNA sequence analysis. DNA sequence analysis is performed using BigDye Terminator v3.1 Cycle Sequencing Kits from Applied Biosystems. Sequencing products are analyzed on a 3730 DNA Analyzer from Applied Biosystems.
Purified DNA was used as template in one or more BioArray Solutions BeadChip™ assays. The *RHCE* and *RHD* BeadChip™ tests for 25 and 35 genetic markers associated with *RHCE* and *RHD* variants, respectively. Data analysis was performed using BASIS 3.3. Genotypes from BeadChip™ and gel-based genotyping assay results were interpreted together to predict the Rh phenotype. Only nucleotides that differ from consensus are listed.

Test Limitations
This testing does not detect polymorphisms other than these assayed, including genetic variants that may result in a null phenotype. Since these methods involve hybridization and PCR amplification with nucleotide primers, other polymorphisms in the primer or probe binding region can affect the testing, and ultimately, the predicted phenotypes. Some of these methods involve restriction enzyme digestion, other polymorphisms in or around the restriction site can affect the testing, and ultimately, the predicted phenotypes. An individual's phenotype may be affected by non-genetic factors. Findings from this testing should be confirmed by another method. In addition, the genotype obtained from DNA isolated from leucocytes and other hematopoietic cells may differ from that of other tissues in persons with a history of transplantation.

Disclaimers
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In some cases, the probable genotype and predicted phenotype takes into account reported serologic antigen typing.
In some cases, the *RHD* zygosity is not determined; this is not expected to impact the predicted phenotype.

Abbreviations
PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; AS, allele-specific PCR; gDNA seq, genomic DNA sequence analysis; bp, base pair; HEA, PreciseType™ HEA Molecular BeadChip or HEA BeadChip™, *RHCE* Array, *RHCE* BeadChip™, *RHD* Array, *RHD* BeadChip™. Gene nucleotides are consecutively numbered from the A in initiating codon (c.A1 etc) and protein products are consecutively numbered from the initiating methionine (p.Met1 etc)

References

Blood Group Antigen FactsBook, 3rd ed. ME Reid and C Lomas-Francis 2012
http://www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology/blood-group-terminology/blood-group-allele-terminology/
http://www.uni-ulm.de/~fwagner/RH/RB2/
Hashmi G *et al. Transfusion* 2005; 45:680-8.

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Report Date: [redacted]

INSTITUTION: [redacted]

Provider [redacted]

SAMPLE: [redacted] (ML20-1793)
ID# [redacted]; DOB: [redacted]

Date received: 07/07/20
Date collected: 07/06/20

SAMPLE TYPE: Whole Blood

HISTORY: African American female, post-partum, G3P2. RhD typing discrepancy - historically D negative, currently testing D positive at hospital (2+ in tube, 3+ in gel).

TESTING REQUESTED: Genotype for *RHD* variants

TESTING PERFORMED			RESULT
***RHD* Variants**	**Method**	**Analyte: Nucleotide (Amino Acid)**	**Nucleotide(s) Detected**
w*RHD* BEADCHIP™	*RHD* Array*	602C>G (T201R)	**G**
		Exon 5 analytes	Low signal**
		1025T>C (I342T)	**C*****

*Only nucleotides which differ from consensus sequence are listed.
**We have found that samples which carry the *RHD*DAR* allele can yield low signal for exon 5 markers on *RHD* BeadChip™.
***RHD c.809 analyte yielded low signal; no impact to phenotype is expected.

Probable *RHD* Genotype: *RHD*DAR* (hemizygous or homozygous)

Predicted phenotype: Partial D+

COMMENTS: *RHD*DAR* alleles are associated with D typing discrepancies and production of allo anti-D.

Females of child bearing potential with partial D should be considered D negative for transfusion and may be candidates for Rh immune globulin (if they have not produced active anti-D).

[redacted] ______________________

[redacted] Laboratory

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Test Methods
DNA was extracted from whole blood on a Qiagen Qiacube, or manually using Qiagen DNA Extraction kits following manufacturer's instructions. Purified DNA was used as template in PCR amplification reactions, some of which were allele-specific reactions to identify single germline variants. Others were PCR-restriction fragment length polymorphism (RFLP) reactions where the PCR product was incubated with a restriction enzyme and the resulting products resolved using agarose gel electrophoresis. Banding patterns are used to interpret the genotype. Some samples are subjected to DNA sequence analysis of one or more exons. In some cases, RNA is isolated from the red cell fraction of the whole blood and used to synthesize cDNA. The cDNA is used as template for gene-specific PCR amplification. In some instances, PCR products are cloned into plasmids prior to DNA sequence analysis. DNA sequence analysis is performed using BigDye Terminator v3.1 Cycle Sequencing Kits from Applied Biosystems. Sequencing products are analyzed on a 3730 DNA Analyzer from Applied Biosystems.
Purified DNA was used as template in one or more BioArray Solutions BeadChip™ assays. The *RHCE* and *RHD* BeadChip™ tests for 25 and 35 genetic markers associated with *RHCE* and *RHD* variants, respectively. Data analysis was performed using BASIS 3.3. Genotypes from BeadChip™ and gel-based genotyping assay results were interpreted together to predict the Rh phenotype. Only nucleotides that differ from consensus are listed.

Test Limitations
This testing does not detect polymorphisms other than these assayed, including genetic variants that may result in a null phenotype. Since these methods involve hybridization and PCR amplification with nucleotide primers, other polymorphisms in the primer or probe binding region can affect the testing, and ultimately, the predicted phenotypes. Some of these methods involve restriction enzyme digestion, other polymorphisms in or around the restriction site can affect the testing, and ultimately, the predicted phenotypes. An individual's phenotype may be affected by non-genetic factors. Findings from this testing should be confirmed by another method. In addition, the genotype obtained from DNA isolated from leucocytes and other hematopoietic cells may differ from that of other tissues in persons with a history of transplantation.

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In some cases, the probable genotype and predicted phenotype takes into account reported serologic antigen typing.
In some cases, the *RHD* zygosity is not determined; this is not expected to impact the predicted phenotype.

Abbreviations
PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; AS, allele-specific PCR; gDNA seq, genomic DNA sequence analysis; bp, base pair; HEA, PreciseType™ HEA Molecular BeadChip or HEA BeadChip™, *RHCE* Array, *RHCE* BeadChip™, *RHD* Array, *RHD* BeadChip™. Gene nucleotides are consecutively numbered from the A in initiating codon (c.A1 etc) and protein products are consecutively numbered from the initiating methionine (p.Met1 etc)

References
Blood Group Antigen FactsBook, 3rd ed. ME Reid and C Lomas-Francis 2012
http://www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology/blood-group-terminology/blood-group-allele-terminology/
http://www.uni-ulm.de/~fwagner/RH/RB2/
Hashmi G *et al. Transfusion* 2005; 45:680-8.

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RHDAX242_3
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Report Date: [redacted]

INSTITUTION: [redacted]   Provider: [redacted]

SAMPLE: [redacted] (ML20-1564CB)
ID# [redacted]; DOB: [redacted]

Date received: 06/16/20
Date collected: 06/12/20

SAMPLE TYPE: Whole Blood

HISTORY: African American female. *RHD* testing was reported 06/22/20. Additional request received 06/23/20.

TESTING REQUESTED: Genotype for C variants

TESTING PERFORMED			RESULT
***RHCE* Common**	**Method**	**Analyte**	**Product present/absent**
***RHCE* gene**	*RHCE* Array	C	absent
		c	present
		Analyte: Nucleotide (Amino Acid)	**Nucleotide(s) Detected**
***RHCE* Exon 5**	*RHCE* Array	676G>C (A226P)	G
***RHCE* Variants**	**Method**	**Analyte: Nucleotide (Amino Acid)**	**Nucleotide(s) Detected**
***RHCE* Exon 2**	RFLP	254C>G (A85G)	C
***wRHCE* BEADCHIP™**	*RHCE* Array*	48G>C (W16C)	**C**
		712A>G (M238V)	**A/G**
		733C>G (L245V)	**G**
		916A>G (I306V)	**A/G**
		1006G>T (G336C)	**G/T**

*Only nucleotides which differ from consensus sequence are listed.

Probable *RHD* Genotype (reported previously): *RHD*DAR / RHD*DIIIa-CE(4-7)-D*

Probable *RHCE* Genotype: *RHCE*RHCE*ce48C,733G,1006T / RHCE*ceAR*

Predicted phenotype: Partial D+ altered C+ E- partial c+ partial e+ VS+V+^{w}

COMMENTS: The patient is at risk for production of allo anti-D, -C, -e, -c or –f (ce).
If transfusion is needed, this patient may benefit from *RH* genotype-matched donors. The American Rare Donor Program may be of assistance in locating Rh compatible units.

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Mitchell Lindquist, MS
Lead Technologist

Margaret A. Keller, PhD
Sr. Director, National Molecular Laboratory

Test Methods

DNA was extracted from whole blood on a Qiagen Qiacube, or manually using Qiagen DNA Extraction kits following manufacturer's instructions. Purified DNA was used as template in PCR amplification reactions, some of which were allele-specific reactions to identify single germline variants. Others were PCR-restriction fragment length polymorphism (RFLP) reactions where the PCR product was incubated with a restriction enzyme and the resulting products resolved using agarose gel electrophoresis. Banding patterns are used to interpret the genotype. Some samples are subjected to DNA sequence analysis of one or more exons. In some cases, RNA is isolated from the red cell fraction of the whole blood and used to synthesize cDNA. The cDNA is used as template for gene-specific PCR amplification. In some instances, PCR products are cloned into plasmids prior to DNA sequence analysis. DNA sequence analysis is performed using BigDye Terminator v3.1 Cycle Sequencing Kits from Applied Biosystems. Sequencing products are analyzed on a 3730 DNA Analyzer from Applied Biosystems.

Purified DNA was used as template in one or more BioArray Solutions BeadChip™ assays. The *RHCE* and *RHD* BeadChip™ tests for 25 and 35 genetic markers associated with *RHCE* and *RHD* variants, respectively. Data analysis was performed using BASIS 3.3. Genotypes from BeadChip™ and gel-based genotyping assay results were interpreted together to predict the Rh phenotype. Only nucleotides that differ from consensus are listed.

Test Limitations

This testing does not detect polymorphisms other than these assayed, including genetic variants that may result in a null phenotype. Since these methods involve hybridization and PCR amplification with nucleotide primers, other polymorphisms in the primer or probe binding region can affect the testing, and ultimately, the predicted phenotypes. Some of these methods involve restriction enzyme digestion, other polymorphisms in or around the restriction site can affect the testing, and ultimately, the predicted phenotypes. An individual's phenotype may be affected by non-genetic factors. Findings from this testing should be confirmed by another method. In addition, the genotype obtained from DNA isolated from leucocytes and other hematopoietic cells may differ from that of other tissues in persons with a history of transplantation.

Disclaimers

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In some cases, the probable genotype and predicted phenotype takes into account reported serologic antigen typing.

In some cases, the *RHD* zygosity is not determined; this is not expected to impact the predicted phenotype.

Abbreviations

PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; AS, allele-specific PCR; gDNA seq, genomic DNA sequence analysis; bp, base pair; HEA, PreciseType™ HEA Molecular BeadChip or HEA BeadChip™, *RHCE* Array, *RHCE* BeadChip™, *RHD* Array, *RHD* BeadChip™. Gene nucleotides are consecutively numbered from the A in initiating codon (c.A1 etc) and protein products are consecutively numbered from the initiating methionine (p.Met1 etc)

References

Blood Group Antigen FactsBook, 3rd ed. ME Reid and C Lomas-Francis 2012
http://www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology/blood-group-terminology/blood-group-allele-terminology/
http://www.uni-ulm.de/~fwagner/RH/RB2/
Hashmi G *et al. Transfusion* 2005; 45:680-8.

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RH0AJ868_2
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Report Date: [redacted]

INSTITUTION: [redacted] Provider: [redacted]

SAMPLE: [redacted] (ML20-1283C)
ID# [redacted]; DOB: [redacted]

Date received: 05/27/20
Date collected: 05/14/20

SAMPLE TYPE: Whole Blood

HISTORY: African American male in sickle cell crisis. Recently transfused.
Anti-E, -Fy^a, -Jk^b, -U, -C^w, -Kp^a, warm autoantibody. RBCs type C+ with possible anti-C.
PreciseType™ HEA Molecular BeadChip testing was reported 05/22/20.

TESTING REQUESTED: Genotype for Rh variants

TESTING PERFORMED			RESULT
***RHD* Variants**	**Method**	**Analyte: Nucleotide (Amino Acid)**	**Nucleotide(s) Detected**
***RHD* Exon 8**	RFLP	1136C>T (T379M)	C
w*RHD* BEADCHIP™	*RHD* Array*	186G>T (L62F)	**G/T**
		410C>T (A137V)	**C/T**
		455A>C (N152T)	**A/C**
***RHCE* Common**	**Method**	**Analyte**	**Product present/absent**
***RHCE* gene**	*RHCE* Array	C	absent
		c	present
		Analyte: Nucleotide (Amino Acid)	**Nucleotide(s) Detected**
***RHCE* Exon 5**	*RHCE* Array	676G>C (A226P)	G
***RHCE* Variants**	**Method**	**Analyte: Nucleotide (Amino Acid)**	**Nucleotide(s) Detected**
***RHCE* Exon 2**	RFLP	254C>G (A85G)	C
w*RHCE* BEADCHIP™	*RHCE* Array*	48G>C (W16C)	**G/C**
		733C>G (L245V)	**G**
		1006G>T (G336C)	**G/T**

*Only nucleotides which differ from consensus sequence are listed.

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Probable *RHD* Genotype: *RHD*01 / RHD*DIIIa-CE(4-7)-D*

Probable *RHCE* Genotype: *RHCE*ce733G / RHCE*ce48C,733G,1006T*

Predicted phenotype: D+ altered C+ E- partial c+ partial e+ VS+V+ $hr^{B}+^{vw}/-$ $hr^{S}+$

COMMENTS: The patient expresses an altered C antigen and is associated with production of allo anti-C. In addition, the patient is at risk for production of allo anti-c, -e and –ce(f). Alloimmunization to hr^{B} is possible. If transfusion is needed, this patient may benefit from *RH* allele selected donors. The American Rare Donor Program may be of assistance in locating such units.

Mitchell Lindquist, MS
Lead Technologist

Margaret A. Keller, PhD
Sr. Director, National Molecular Laboratory

Test Methods
DNA was extracted from whole blood on a Qiagen Qiacube, or manually using Qiagen DNA Extraction kits following manufacturer's instructions. Purified DNA was used as template in PCR amplification reactions, some of which were allele-specific reactions to identify single germline variants. Others were PCR-restriction fragment length polymorphism (RFLP) reactions where the PCR product was incubated with a restriction enzyme and the resulting products resolved using agarose gel electrophoresis. Banding patterns are used to interpret the genotype. Some samples are subjected to DNA sequence analysis of one or more exons. In some cases, RNA is isolated from the red cell fraction of the whole blood and used to synthesize cDNA. The cDNA is used as template for gene-specific PCR amplification. In some instances, PCR products are cloned into plasmids prior to DNA sequence analysis. DNA sequence analysis is performed using BigDye Terminator v3.1 Cycle Sequencing Kits from Applied Biosystems. Sequencing products are analyzed on a 3730 DNA Analyzer from Applied Biosystems.

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Disclaimers
The PreciseType™ HEA Molecular BeadChip Test has been cleared by the Food and Drug Administration (FDA) as an *in vitro* diagnostic test intended for the molecular determination of allelic variants that predict erythrocyte antigen phenotypes in the Rh, Kell, Duffy, Kidd, MNS, Lutheran, Dombrock, Landsteiner-Wiener, Diego and Scianna blood group systems in human genomic DNA.

All other tests performed by The National Molecular Laboratory are for research use only. These tests were developed and their performance characteristics established by The National Molecular Laboratory. The tests have not been cleared or approved by the Food and Drug Administration (FDA) or other regulating bodies and are, therefore, not FDA-licensed tests. The National Molecular Laboratory participates in proficiency programs for this testing. These results are intended for predicting blood group or platelet antigens in patients and donors, and are not intended for clinical diagnosis or as the sole means for patient management decisions.

In some cases, the probable genotype and predicted phenotype takes into account reported serologic antigen typing.

Abbreviations
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Report Date: 06/13/19

INSTITUTION: [redacted] Physician: Dr. [redacted]

SAMPLE: [redacted] (ML19-1439)
Patient ID#: [redacted]; DOB: [redacted]

Date received: 06/05/19
Date collected: 06/03/19

SAMPLE TYPE: Whole Blood

HISTORY: Asian female, prenatal. Patient types Jk(a-b-) and has a probable anti-Jk3. PreciseType™ HEA Molecular BeadChip testing was reported on 06/12/19.

TESTING REQUESTED: Genotype for Kidd *(JK)* variants.

TESTING PERFORMED: Amplification and sequencing of all *JK* coding exons (4 through 11) and their respective splice sites.

RESULTS:

Gene or Region	Method	Analyte	Result	Interpretation
			Nucleotides detected*	**Predicted Amino Acid**
JK	gDNA seq*	Exon 4	No changes from consensus	
		Exon 5	No changes from consensus	
		Exon 6	**342-1g**	**N/A**
		Exon 7	588G	**N/A**
		Exon 8	No changes from consensus	
		Exon 9 (*JK*A/JK*B*)	838A	280N
		Exon 10	No changes from consensus	
		Exon 11	No changes from consensus	

*Only nucleotides which differ from consensus sequence are listed.

Probable Genotype: *JK*02N.01 / JK*02N.01*

Predicted Phenotype: Jk(a-b-)

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COMMENTS: Genomic sequencing of all coding exons of *JK* identified the intragenic c.342-1G>A polymorphism, which causes alternative splicing of exon 6 and a null JK phenotype.

Supervisor, National Molecular Laboratory

Manager, National Molecular Laboratory

Test Methods
DNA was extracted from whole blood on a Qiagen Qiacube, or manually using Qiagen DNA Extraction kits following manufacturer's instructions. Purified DNA was used as template in PCR amplification reactions, some of which were allele-specific reactions to identify single germline variants. Others were PCR-restriction fragment length polymorphism (RFLP) reactions where the PCR product was incubated with a restriction enzyme and the resulting products resolved using agarose gel electrophoresis. Banding patterns are used to interpret the genotype. Some samples are subjected to DNA sequence analysis of one or more exons. In some cases, RNA is isolated from the red cell fraction of the whole blood and used to synthesize cDNA. The cDNA is used as template for gene-specific PCR amplification. In some instances, PCR products are cloned into plasmids prior to DNA sequence analysis. DNA sequence analysis is performed using BigDye Terminator v3.1 Cycle Sequencing Kits from Applied Biosystems. Sequencing products are analyzed on a 3730 DNA Analyzer from Applied Biosystems.

Test Limitations
This testing does not detect polymorphisms other than these assayed, including genetic variants that may result in a null phenotype. Since these methods involve hybridization and PCR amplification with nucleotide primers, other polymorphisms in the primer or probe binding region can affect the testing, and ultimately, the predicted phenotypes. Some of these methods involve restriction enzyme digestion, other polymorphisms in or around the restriction site can affect the testing, and ultimately, the predicted phenotypes. An individual's phenotype may be affected by non-genetic factors. Findings from this testing should be confirmed by another method. In addition, the genotype obtained from DNA isolated from leucocytes and other hematopoietic cells may differ from that of other tissues in persons with a history of transplantation.

Disclaimers
The PreciseType™ HEA Molecular BeadChip Test has been cleared by the Food and Drug Administration (FDA) as an *in vitro* diagnostic test intended for the molecular determination of allelic variants that predict erythrocyte antigen phenotypes in the Rh, Kell, Duffy, Kidd, MNS, Lutheran, Dombrock, Landsteiner-Wiener, Diego and Scianna blood group systems in human genomic DNA.

All other tests performed by The National Molecular Laboratory are for research use only. These tests were developed and their performance characteristics established by The National Molecular Laboratory. The tests have not been cleared or approved by the Food and Drug Administration (FDA) or other regulating bodies and are, therefore, not FDA-licensed tests. The National Molecular Laboratory participates in proficiency programs for this testing. These results are intended for predicting blood group or platelet antigens in patients and donors, and are not intended for clinical diagnosis or as the sole means for patient management decisions.

In some cases, the probable genotype and predicted phenotype takes into account reported serologic antigen typing.

Abbreviations
PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; AS, allele-specific PCR; gDNA seq, genomic DNA sequence analysis; bp, base pair; HEA, HEA BeadChip™ or PreciseType™ HEA Molecular BeadChip, *RHCE* Array, *RHCE* BeadChip, *RHD* Array, *RHD* BeadChip. Gene nucleotides are consecutively numbered from the A in initiating codon (c.A1 etc) and protein products are consecutively numbered from the initiating methionine (p.Met1 etc)

References

1. http://www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology/blood-group-terminology/blood-group-allele-terminology/
2. Whorley *et al. Transfusion* 49:3S 48A-49A, 2009.
3. Wester *et al. Transfusion* 51: 380-392, 2011.

Internal Use Only:

Report Prepared by RG 06/12/19
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