HEMO ID - BLOOD GROUP GENOTYPING USING MALDI-TOF MS
From Idea to Panel

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Agena Bioscience GmbH, Hamburg
Presentation outline

- Need to know about Blood groups and blood transfusion
- MassARRAY® System
  - MALDI-TOF Mass Spectrometry
  - iPLEX® Pro Biochemistry using Hemo ID
- Hemo ID™ Blood Group Typing Panel
  - Modules
  - RH D Zygosity
- Hemo ID in Research
- Perspective
History of Transfusion Medicine

- In 1628 British physician William Harvey discovers the circulation of blood in human body
- 1665 Richard Lower conducts the first recorded case of transfusion, dog to dog
- 1818 James Blundell conducts the first recorded case of successful human blood transfusion
- 1901 Karl Landsteiner discovers ABO blood groups
- 1907 Reuben Ottenberg performs the first blood transfusion using blood typing and cross-matching.
- 1939 – 1940 Karl Landsteiner, Alexander Wiener, Philip Levine and R.E. Stetson discovered the Rh blood group system
- 2007 Molecular genotyping becomes a commonly used tool for blood group typing
What are Blood Groups?

- Different types of Antigens on the Surface of Red Blood Cells (RBC)
Why are Blood Types so Important?

- Different types of Antigens on the Surface of Red Blood Cells for self identification
- Antibodies can be found in Blood Plasma to fight off foreign blood groups, resulting in co-aggulation
Multiple Blood Groups per Individual

A                 RhD             kk

O                 Rhd             KK
Co-expression of Blood Group Antigens

- Currently, 34 major blood group systems recognized including the ABO, Rhesus and Kell systems.
- In addition to the ABO, Rhesus and Kell antigens, up to 200 antigens (minor blood group systems) are co-expressed on the red blood cell surface membrane.
- E.g. an individual can be AB, RhD positive, and K positive (Kell system), and at the same time M or N positive (MNS system), and Le\textsuperscript{a} or Le\textsuperscript{b} positive (Lewis system), etc.
How donated blood is used

- 1 in 3 people will need blood or blood products once in their lives.

The red cells from your donation are used in the following ways:

- 34% Cancer and blood diseases
- 19% Other causes of anaemia
- 18% Surgical patients including open heart surgery and burns
- 13% Other medical problems including heart, stomach and kidney disease
- 10% Orthopaedic patients including fractures and joint replacements
- 4% Obstetrics, including pregnant women, new mothers and young children
- 2% Trauma including road accidents

Source: Bloodhound Study (ARCBS and Monash Institute of Health Services Research) 2007
How donated blood is used

- 1 in 3 people will need blood or blood products once in their life

In EU 5% of population donates blood every year

On average 25 million blood donations are made per year in Europe
In most countries, donated blood is tested for infectious diseases, and typed for ABO and Rhesus.

- Hepatitis A virus
- Hepatitis B virus
- Hepatitis C virus
- HIV Types 1
- HIV Type 2
- Treponema pallidum (Syphilis)
- Human T-Lymphotropic Virus, Types I
- Human T-Lymphotropic Virus, Type II
- West Nile Virus
- Trypanosoma cruzi (Chagas disease)

- ABO, Rhesus matching is sufficient for almost all one-time transfusions

- 107 million units of blood are donated yearly in the world
- Level of testing depends upon available resources and disease prevalence
What happens in multiple transfusions?

ABO and rhesus matching is inadequate in multiple transfusions

Donor red cells contain wild type antigen

Recipient’s red cells have a variant antigen

Recipient’s immune system responds to wild type antigen

Repeated encounters with wild type molecules on donor RBCs

Recipient’s antibodies destroy donor’s RBCs resulting in life-threatening immediate or delayed hemolytic reaction
Serological testing has been the gold standard for detecting rare blood antigens

The classical method for recipient/donor matching

Robust antibodies are available for only 18 of more than 200 rare blood antigens

<table>
<thead>
<tr>
<th>Antisera</th>
<th>Reagent cost/ Test</th>
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<tbody>
<tr>
<td>C</td>
<td>0.95 -1.15</td>
</tr>
<tr>
<td>E</td>
<td>0.95 -1.15</td>
</tr>
<tr>
<td>c</td>
<td>0.95 - 1.15</td>
</tr>
<tr>
<td>e</td>
<td>2.20 - 3.68</td>
</tr>
<tr>
<td>M</td>
<td>3.56 - 4.63</td>
</tr>
<tr>
<td>N</td>
<td>3.74 - 4.91</td>
</tr>
<tr>
<td>S</td>
<td>7.18 - 18.79</td>
</tr>
<tr>
<td>s</td>
<td>3.18 - 8.63</td>
</tr>
<tr>
<td>K</td>
<td>1.07 - 1.55</td>
</tr>
<tr>
<td>Fya</td>
<td>2.58 - 7.11</td>
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<tr>
<td>Fyb</td>
<td>9.18 - 11.43</td>
</tr>
<tr>
<td>Jka</td>
<td>4.63 - 12.31</td>
</tr>
<tr>
<td>Jkb</td>
<td>4.98 - 13.21</td>
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<tr>
<td>Dia</td>
<td>2.68</td>
</tr>
<tr>
<td>Kpa</td>
<td>2.24</td>
</tr>
<tr>
<td>Kpb</td>
<td>2.24</td>
</tr>
<tr>
<td>Lub</td>
<td>3.20</td>
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<tr>
<td>k</td>
<td>5.13</td>
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<tr>
<td>Manual testing reagent cost</td>
<td>$100</td>
</tr>
<tr>
<td>Labor, controls, disposables</td>
<td>$50</td>
</tr>
<tr>
<td><strong>Cost for one sample</strong></td>
<td><strong>$150</strong></td>
</tr>
</tbody>
</table>
MassARRAY® System

- Nanodispenser robot (RS1000)
- Desktop mass spectrometer (MassARRAY® Mass Analyzer 4)
- Database with software
How Does MALDI-TOF Work?
Matrix Assisted Laser Desorption Ionization Time Of Flight Mass Spectrometry (MALDI-TOF MS)

Laser
Matrix/Analyte
Detector

Mass Spectrum m/z
iPlex® Pro Genotyping Assay

10 mer tag

A

10 mer tag

G

10 mer tag

A

10 mer tag

G

10 mer tag

A

10 mer tag

G

10 mer tag

A

10 mer tag

G

Sample Conditioning, nanodispensing on a SpectroCHIP and measurement on MA4
**MassARRAY® Advantages**

- Combines sensitivity of PCR with accuracy of Mass Spectrometry
- High-level multiplexing PCR (e.g. Kell-Kidd-Duffy 17plex/reaction)
- Direct analysis of molecular mass of molecule of interest
- No special primer, no label
- Same plate from start to finish
MassARRAY® System - Applications

- Genotyping
- Methylation Analysis
- Somatic Mutation Detection
- Blood Group Typing
- Gene Expression
- Copy Number Variation
- Oligo Check
- Comparative Sequence Analysis
From Hemo ID ABS prototype to final RUO Hemo ID Panel

Sources of contribution and feedback

- 2010: co-operational project with Christoph Gassner, BSD Zurich
  - Prototype for proof of principle → system deal in August 2011
- Customized single multiplex solution for German Red Cross, Hagen
  - Own system since March 2012
- American Red Cross, Philadelphia
  - Own system since May 2014
- Diverse Hemo ID ABS service studies, a.o.
  - Australian Red Cross
  - UK Reference Laboratory Filton, UK
  - Établissement Français du Sang, France
- External reviewers for module definition software files
- Launch of Hemo ID panel in April 2014
Hemo ID Blood Group Genotyping Panel

101 antigens or 167 alleles in 108 SNP assays across 17 different blood group, platelet and granulocyte systems

- Comprehensive analysis of 76 samples, or less complex analyses of 768 samples in 8 hrs (8 chips/day)
- Includes newly discovered variants (Vel-) and unique copy number variation assays/Rhesus D zygosity
- Kit format: six different combinations of assays (Modules)
- High sensitivity and accuracy of mass spectrometry
- Hemo ID software for genotype analysis and generation of ‘predicted phenotypes’- easy custom assay plug-in
- Assays by Agena Bioscience service for custom assays
HemoID - Which blood groups can be tested?

- Rhesus D, Rhesus C and Rhesus E status, RHD weak types, RHDel, other Rhesus hybrids and variants
- Kell
- Kidd
- Duffy
- MNS
- Indian
- Lutheran
- Auburger
- Landsteiner-Wiener
- Diego
- Colton
- Knops
- Dombrock
- Scianna
- Cartwright
- Vel
HemoID - Which blood groups can be tested?

- Rhesus D, Rhesus C and Rhesus E status, RHD weak types, RHDel, other Rhesus hybrids and variants
- Landsteiner-Wiener
- Kell
- Diego
- Kidd
- Colton
- Duffy
- Knops
- MNS
- Dombrock
- Indian
- Scianna
- Lutheran
- Cartwright
- Auberger
- Vel
- Plus:
  - HPA
  - HNA
High flexibility achieved through a modular design

108 SNP assays covering 101 antigens or 167 alleles across 17 different blood group, platelet and granulocyte systems

<table>
<thead>
<tr>
<th>10 Multiplex Reactions in 6 Modules</th>
<th>Phenotypes</th>
<th>Multiplex Reactions</th>
<th>Plex level/well</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Kell, Kidd, Duffy</td>
<td>18</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>2 MNS</td>
<td>6</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>3 Rare Blood Groups</td>
<td>40</td>
<td>1 of 2</td>
<td>17</td>
</tr>
<tr>
<td>4 RHD/CE Broad</td>
<td>20</td>
<td>1 of 3</td>
<td>16</td>
</tr>
<tr>
<td>5 RHD/CE Variant</td>
<td>37</td>
<td>1 of 2</td>
<td>14</td>
</tr>
<tr>
<td>6 HPA &amp; HNA</td>
<td>13</td>
<td>1</td>
<td>14</td>
</tr>
</tbody>
</table>
# Three Blood groups covered by one single multiplex reaction

including null variant detection of compound heterozygous samples

<table>
<thead>
<tr>
<th>Blood Group</th>
<th>Variant / SNP ID</th>
</tr>
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<tbody>
<tr>
<td><strong>Kell</strong></td>
<td></td>
</tr>
<tr>
<td>Kell / Cellano</td>
<td>KEL*01/*02 (M193T)</td>
</tr>
<tr>
<td>Kp&lt;sub&gt;a&lt;/sub&gt; / Kp&lt;sub&gt;b&lt;/sub&gt;</td>
<td>KEL<em>02.03/KEL</em>02 (W281R)</td>
</tr>
<tr>
<td>Js&lt;sub&gt;a&lt;/sub&gt; / Js&lt;sub&gt;b&lt;/sub&gt;</td>
<td>KEL<em>02.06/KEL</em>02 (P597L)</td>
</tr>
<tr>
<td>K&lt;sub&gt;mod&lt;/sub&gt;</td>
<td>KEL*02M.05 (G573G)</td>
</tr>
<tr>
<td>K&lt;sub&gt;0&lt;/sub&gt;</td>
<td>KEL*02N.01 (IVS3+1G&gt;C)</td>
</tr>
<tr>
<td>K&lt;sub&gt;0&lt;/sub&gt;</td>
<td>KEL*02N.02 (R128X)</td>
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<tr>
<td>K&lt;sub&gt;0&lt;/sub&gt;</td>
<td>KEL*02N.04 (Q348X)</td>
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<td>KEL*02N.06 (IVS3+1G&gt;A)</td>
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<td>KEL*02N.12 (IVS8+1G&gt;A)</td>
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<td>KEL*02N.13 (IVS8+1G&gt;T)</td>
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<tr>
<td>K&lt;sub&gt;0&lt;/sub&gt;</td>
<td>KEL*02N.17 (R516X)</td>
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<tr>
<td><strong>Jk&lt;sub&gt;a&lt;/sub&gt; / Jk&lt;sub&gt;b&lt;/sub&gt;</strong></td>
<td>Jk&lt;sub&gt;0&lt;/sub&gt;</td>
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<tr>
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<tr>
<td>JK&lt;sub&gt;0&lt;/sub&gt;</td>
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<tr>
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<td>FY*01/*02 (G42D)</td>
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<tr>
<td>Fy&lt;sub&gt;0&lt;/sub&gt; (GATA)</td>
<td>FY*01N.01 (P-67T&gt;C)</td>
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<tr>
<td>Fy&lt;sub&gt;b&lt;/sub&gt; / Fy&lt;sub&gt;x&lt;/sub&gt;</td>
<td>FY*02M (R89C)</td>
</tr>
</tbody>
</table>

*customized RUO product available through Assays by Sequenom*
SNP - aminoacid change of N-glycosylation site: Cellano (KEL2) => Kell (KEL1)
SNP - aminoacid change of N-glycosylation site: Cellano (KEL2) => Kell (KEL1)

Amplification primer

TTTAACCGAA CGCTGATGGATCAG

TTTAACCGAA CGCTGATGGATCAG

**KEL*02/02 (kk)**

TTTAACCGAA CGCTGATGGATCAG

TTTAACCGAA TGCTGATGGATCAG

**KEL*01/02 (Kk)**

TTTAACCGAA TGCTGATGGATCAG

TTTAACCGAA TGCTGATGGATCAG

**KEL*01/01 (KK)**

Amplification primer
SNP - aminoacid change of N-glycosylation site: Cellano (KEL2) => Kell (KEL1)

Amplification primer

TTTAACCGAAAACGCTGATGGGATCAG

TTTAACCGAAAACGCTGATGGGATCAG

*KEL*02/02 (kk)

CGACTACCTAGTC extension primer

TTTAACCGAAAACGCTGATGGGATCAG

CGACTACCTAGTC extension primer

*KEL*01/02 (Kk)

TTTAACCGAAAACGCTGATGGGATCAG

*KEL*01/01 (KK)
SNP - amino acid change of N-glycosylation site: Cellano (KEL2) => Kell (KEL1)

Amplification primer
TTTAACCGAA\textcolor{red}{C}GCTGATGGGATCAG\
TTTAACCGAA\textcolor{red}{C}GCTGATGGGATCAG

\textit{KEL}^{*02/02} (kk)

\textcolor{red}{CG}A\textcolor{red}{C}G\textcolor{red}{A}C\textcolor{red}{T}A\textcolor{red}{C}C\textcolor{red}{T}\textcolor{red}{A}\textcolor{red}{G}\textcolor{red}{T}C extension primer, 5200 Da

\textcolor{red}{CG}A\textcolor{red}{C}G\textcolor{red}{A}C\textcolor{red}{T}A\textcolor{red}{C}C\textcolor{red}{T}\textcolor{red}{A}\textcolor{red}{G}\textcolor{red}{T}C extension primer, 5216 Da

\textit{KEL}^{*01/02} (Kk)

\Delta_{(A \text{ vs. } G)} = 16\text{Da}

\textit{KEL}^{*01/01} (KK)
Spectra of three different Jsa/Jsb (Suter or KEL6/KEL7) DNAs

- MALDI-TOF MS measures molecular weight (in Dalton) of extension primers (5612) plus 1 single base extension.
- Heterozygous samples show 2 different molecular weight peaks at about 5882 and 5898 Dalton (all indicated by arrow).
- Other peaks are results of other bloodgroup SNPs, detected in the same multiplex PCR.
Δ(A vs. G) = 16 Da

Spectra of three different Jsa/Jsb (Suter or KEL6/KEL7) DNAs

Primer unextended 5612 Da
Primer extended 5882 Da 5898 Da

KEL_3_1

- No Call (25)
- T (161)
- TC (5)
- C (1)
- Other (25)
Spectra of three different Jsa/Jsb (Suter or KEL6/KEL7) DNAs

Δ(A vs. G) = 16 Da

Primer unextended
5612 Da

Primer extended
5882 Da  5898 Da
Hemo ID Blood Group Genotyping Panel Reporting Software

- Provides ISBT genotypes, predicted ISBT phenotypes and traditional phenotypes for >100 blood antigens
- Allows the user to define custom phenotypes for inclusion in reports
- Provides reports in both CSV format for integration in LIM systems and HTML format for rich graphical display and viewing
- Allows the user to include a custom logo to be displayed in the HTML report header
- Supports printing reports with smart pagination and signature/date blocks.
Customization - Hemo ID Report Settings Panel

Sequenom Hemo ID Panel Settings

Language Options
- Current: English
  Available languages...

Reference Phenotype
- Phenotype (ISBT)
- Phenotype (Traditional)
- Phenotype (Custom)
- Genotype (ISBT)
- Genotype

Default Display Options
- Assay and Alleles
- Genotype
- Genotype (ISBT)
- Phenotype (ISBT)
- Phenotype (Traditional)
- Phenotype (Custom)
- Plots

Reporting Options
- Generate Plots
- Low Call Rate Threshold (%)

Select a file containing custom phenotypes

Select a logo to display in the reports

Save
Multiple Report Options

- One sample – one module
- Full Phenotype report per full sample set

Complete Report: HNA, HPA

<table>
<thead>
<tr>
<th>Blood Group or Antigen</th>
<th>Assay</th>
<th>Genotype</th>
<th>Genotype (ISBT)</th>
<th>Predicted Phenotype (ISBT)</th>
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<tbody>
<tr>
<td>HNA1</td>
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<td>HET</td>
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<td></td>
<td>HNA1_b [A G]</td>
<td>HET</td>
<td>SLC44A2*01</td>
<td></td>
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<tr>
<td></td>
<td>HNA1_c [C A]</td>
<td>C</td>
<td>SLC44A2*01</td>
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<tr>
<td>HNA3_1</td>
<td>HNA3_1 [G A]</td>
<td>GG</td>
<td>SLC44A2*01</td>
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<td>HNA4_1 [A G]</td>
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<td>ITGAM*2358</td>
<td>HNA-4abw</td>
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<td>HNA5_1 [G G]</td>
<td>CG</td>
<td>ITGAL*2372</td>
<td>HNA-5abw</td>
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<tr>
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<td>TT</td>
<td>ITGB3*001</td>
<td>HPA-1a</td>
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<tr>
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<td>HPA2_1 [C T]</td>
<td>CC</td>
<td>ITGB3*001</td>
<td>HPA-2a</td>
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<td>ITGB3*001</td>
<td>HPA-3a</td>
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<td>ITGB3*001</td>
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<td>HPA5_1</td>
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<td>GG</td>
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<tr>
<td>HPA15_1</td>
<td>HPA15_1 [C A]</td>
<td>CC</td>
<td>ITGB3*001</td>
<td>HPA-15a</td>
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</tbody>
</table>
RHD Exon Screening Plots
"The observed success rates, data quality and concordance with known blood group types are highly impressive, underlining the accuracy and reliability of this cost-efficient high throughput method."

Dr. Christoph Gassner – Blood Transfusion Service, Zurich
High-throughput Kell, Kidd, and Duffy matrix-assisted laser desorption/ionization, time-of-flight mass spectrometry–based blood group genotyping of 4000 donors shows close to full concordance with serotyping and detects new alleles

Stefan Meyer,¹* Caren Vollmert,²* Nadine Trost,¹ Chantal Brönnimann,¹ Jochen Gottschalk,³ Andreas Buser,⁴ Beat M. Frey,³ and Christoph Gassner¹
Performance comparison

- AbS determined Kell, Kidd and Duffy genotypes with HemoCarta Oligo Set, and manually obtained predicted phenotypes in Zurich (4000). Sequenom Hamburg (760) used HemoCarta Oligo Set. Data analysis was done manually and using the HemoID software
  - Obtained 100% concordance with Zurich genotyping

- Sequenom San Diego used Hemo ID Panel (760) and HemoID software and found 100% concordance with Hamburg and Zurich genotyping, therefore HemoID also had 100% concordance with genotyping for Kell, Kidd, Duffy

Meyer S., Vollmert C. et al. (2014) “High-throughput Kell, Kidd and Duffy MALDI-TOF MS based blood group genotyping of 4,000 donors shows close to full concordance with serotyping and detects new alleles.” Transfusion, in press
We started the CE / CE/IVD process directly after launch and expect to obtain approval within 2014.

Further development in progress:
- MNS Module with more variants
Hemo ID Blood Group Genotyping Panel

**The Science of Predicting Phenotypes**

- **167 Alleles**
- 16 blood group systems
- 6 Modules

**UNMATCHED DEPTH OF ANALYSIS**

Type 101 antigens in 16 blood group systems, and 23 platelet (HPA) and neutrophil antigens (HNA). Use the complete Hemo ID Panel for a comprehensive analysis, or select Hemo ID Modules for focused analyses.

**PRECISION OF MASS SPECTROMETRY**

Benefit from the accuracy, precision, and throughput of the MassARRAY® System – a proven genetic analysis technology referenced in over 2,000 scientific publications.

**FLEXIBLE THROUGHPUT**

Obtain predicted phenotypes from as many as 3,000 samples in Full Flex, and reliably identify samples negative for high incidence antigens as well as those positive for low incidence antigens.

**COMPREHENSIVE RH GENOTYPING**

Analyze complex RH genotypes and identify variants with allele-specific MHD typing, D, weak and partial MHD alleles, and MHC-HLA hybrid alleles.

**AUTOMATED GENOTYPE TO PREDICTED PHENOTYPE CONVERSION**

Select from multiple user-friendly views of predicted phenotypes, and export formats, including the latest DBT designations.

**AN OPEN GENETIC ANALYSIS PLATFORM**

Easily extend the Hemo ID panel with additional assays for blood variant profiles prevalent in your research population.

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A panel of SNP and CNV assays for use in genotyping 16 blood group systems, and HPA and HNA.

<table>
<thead>
<tr>
<th>Module Name</th>
<th>CAT No.</th>
<th>MSR-ID</th>
<th>PROTOCOL/PHENOTYPES ASSAYED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemo ID® Blood Group Genotyping Panel</td>
<td>1950.00</td>
<td>1952</td>
<td>Includes all six Modules below for a comprehensive analysis</td>
</tr>
<tr>
<td>Hemo ID® Kidd, KEL, KEL+ Module</td>
<td>1950.00</td>
<td>1950</td>
<td>Kidd: K, KEL, KEL+ (KEL) erythrocyte, KEL, KEL+ (erythrocyte, KEL), KEL+ (erythrocyte)</td>
</tr>
<tr>
<td>Hemo ID® Duffy, ABO+Module</td>
<td>1950.00</td>
<td>1950</td>
<td>Duffy: Fy(a+), Fy(a), Fy(a-), Fy(b-), Fy(b)</td>
</tr>
<tr>
<td>Hemo ID® MHC Module</td>
<td>1950.00</td>
<td>1952</td>
<td>MHC: DR, DQ, HLA-A, HLA-B, HLA-C, HLA-D, HLA-E, HLA-F, HLA-G, HLA-H, HLA-L, HLA-M, HLA-N, HLA-P, HLA-Q, HLA-T, HLA-U, HLA-W, HLA-X, HLA-Y, HLA-Z</td>
</tr>
<tr>
<td>Hemo ID® Rh Blood Groups (Rh) Module</td>
<td>1950.00</td>
<td>1950</td>
<td>Rh: RhD, RhC, RhE, RhF, RhG, RhH, RhK, RhL, RhM, RhN, RhO, RhP, RhQ, RhS, RhT, RhU, RhV, RhW, RhX, RhY, RhZ</td>
</tr>
</tbody>
</table>

* The Hemo ID® Blood Group Genotyping Panel is for use in research and only for research use. The system is for the identification of blood group antigens and not for the identification of blood group antigens as a tool for diagnostic use by the W.H.O. or the Y.S.S.G. (World Health Organization of the United Nations).
THANKS FOR YOUR ATTENTION!

Beatrice.Oelze@agena.bio.com
**RHD vs. RHC/E**  
Copy number analysis  
Detection of hybrids, partial D, weak D, Del variants

Signal ratio RH D/CE = 0  => Rh dd samples  
Signal ratio RH D/CE = 0.5  => Rh Dd samples  
Signal ratio RH D/CE = 1  => Rh DD samples
How many samples can I run per day?

This depends on your system configuration and the number of multiplex reactions you are planning to run. Each system can run 8 chips per 8 hour day.

- **96 System at full capacity:**
  
  1 module = 1 reaction: 768 samples
  
  All modules = 10 reactions: 76 samples

- **384 System at full capacity:**
  
  1 module = 1 reaction: 3072 samples
  
  All modules = 10 reactions: 307 samples

- **Example for minimum on 96 system:**
  
  1 module = 1 reaction: 96 samples
  
  All modules = 10 reactions: 9 sample
How many specificities are covered?

- 101 antigens or
- 167 alleles in 104 SNP assays
- across 17 different blood group, platelet and granulocyte systems
- in 6 modules

<table>
<thead>
<tr>
<th>Blood Group System</th>
<th>Σ Plexlevel</th>
<th># of Multiplexes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Kell, Kidd, Duffy</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>2. MNS</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>3. RHD/C/E broad</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>4. RHD/C/E variant</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>5. Rare Blood Groups</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>6. HPA &amp; HNA</td>
<td>14</td>
<td>1</td>
</tr>
</tbody>
</table>

∑ = 10
So far unmet Blood Group Typing Requirements

Where Hemo ID Panel makes the difference

- **Flexibility** to select smaller sets of rare blood cell antigen assays to perform focused testing, and thereby save money
- More comprehensive assay content including the latest discoveries in blood groups, e.g. Vel-
- Detailed analysis of compound heterozygous samples, *RHD* copy number and Rhesus hybrids
- Possibility to include assays for variants with high frequencies in local ethnic populations, and to adapt analysis and reporting software to these additional assays
- **Throughput** - Requirement of screening few as well as 100s to 1000s of DNA samples per day
Immunohematologists know that blood groups are quite complex!

### Major Blood Groups

- Rh
- Kidd
- Diego
- Colton
- Kx
- Kidd
- MNS
- Diego
- Colton

### Minor Blood Groups

There are 34 blood group systems according to the International Society of Blood Transfusion (ISBT). Each blood group system can have different variants.

<table>
<thead>
<tr>
<th>Blood Group System</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rh</td>
<td>JMH</td>
</tr>
<tr>
<td>Kidd</td>
<td>MNS</td>
</tr>
<tr>
<td>Diego</td>
<td>Gerbich</td>
</tr>
<tr>
<td>Colton</td>
<td>Chido/Rodgers</td>
</tr>
<tr>
<td>Kx</td>
<td>Cromer</td>
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<tr>
<td>GIL</td>
<td>Knops</td>
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<tr>
<td>Lutheran</td>
<td>Kell</td>
</tr>
<tr>
<td>Duffy</td>
<td>Yt</td>
</tr>
<tr>
<td>Xg</td>
<td>Dombrock</td>
</tr>
<tr>
<td>Scianna</td>
<td>ABO</td>
</tr>
<tr>
<td>Landsteiner P</td>
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</tr>
<tr>
<td>Wiener</td>
<td>Lewis</td>
</tr>
<tr>
<td>Indian</td>
<td>H</td>
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<tr>
<td>OK</td>
<td>I</td>
</tr>
<tr>
<td>RAPH</td>
<td>GLOB</td>
</tr>
</tbody>
</table>

Minor blood groups are also called “rare antigens.”