



Characterization of 108 Genomic DNA Reference Materials for 11 Human Leukocyte Antigen Loci



A GeT-RM Collaborative Project

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The highly polymorphic human leukocyte antigen (HLA) genes, located in the human major histocompatibility complex, encode the class I and II antigen-presenting molecules, which are centrally involved in the immune response. HLA typing is used for several clinical applications, such as transplantation, pharmacogenetics, and diagnosis of autoimmune disease. HLA typing is highly complex because of the homology of HLA genes and pseudogenes and the extensive polymorphism in the population. The Centers for Disease Control and Prevention established the Genetic Testing Reference Materials Coordination Program (GeT-RM) in partnership with the genetics community to improve the availability of genomic DNA reference materials necessary for quality assurance of genetic laboratory testing. The GeT-RM together with three clinical laboratories and the Coriell Cell Repositories have characterized genomic DNA obtained from a panel of 108 cell lines for all HLA classic polymorphic loci: *HLA-A, B, C, DRB1, DRB3, DRB4, DRB5, DQA1, DQB1, DPA1, and DPB1*. The goal was to develop a publicly available and renewable source of well-characterized genomic DNA reference materials to support molecular HLA typing assay development, validation, and verification, quality control, and proficiency testing. These genomic DNA samples are publicly available from the National Institutes of General Medical Science Repository at the Coriell Cell Repositories. (*J Mol Diagn* 2018, 20: 703–715; <https://doi.org/10.1016/j.jmoldx.2018.05.009>)

The human major histocompatibility complex (MHC), located on the short arm of chromosome 6 at 6p21.3, contains a set of highly polymorphic genes, the human leukocyte antigen (HLA) loci.¹ These genes encode the class I and II molecules (glycoproteins) that bind and present peptide antigens to lymphocyte receptors. Class I molecules, expressed on the surface of all nucleated cells, present peptides derived from intracellular proteins to cytotoxic CD8 T cells and thus participate in the defense against cancer, viruses, and other intracellular parasites. Class I molecules are also ligands of natural killer (NK) cell

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immunoglobulin-like receptors (KIRs). Class II molecules, expressed mainly on the surface of professional antigen-presenting cells, present peptides derived from extracellular proteins to CD4 T cells and thus participate in the defense against extracellular pathogens.²

HLA class I molecules are heterodimeric glycoproteins that consist of an α chain coded by the *HLA-A*, *B*, and *C* genes and of β_2 -microglobulin encoded on chromosome 15. Sequence variation is concentrated primarily in exons 2 and 3 that code for the α_1 - and α_2 -domains of the class I molecules. Peptide antigens interact and bind to these two domains and form a complex; the molecular entity formed is recognized by the T-cell receptor. The edge of the binding cleft formed by the α_1 - and α_2 -domains is also recognized by the KIR. Class II molecules are heterodimers of an α and a β chain. The most polymorphic class II loci are *HLA-DRB1*, *DQB1*, and *DPB1*, which code for the β chain of the DR, DQ, and DP molecules, respectively, and, to a lesser extent, *HLA-DQA1* and *DPA1*, which code for the corresponding α chains. Sequence variation is concentrated on exon 2 that codes for the α_1 - and for the β_1 -domain that constitute the peptide and T-cell receptor binding site. Adding to the genetic variability of the class II region, the DR region can contain one or two *DRB* genes. The *DRB1* gene is present in all haplotypes, and a second *DRB* gene, *DRB3*, *DRB4*, or *DRB5*, is present in only some haplotypes.³

HLA typing, the determination of a particular set of HLA alleles carried by an individual, has high clinical relevance. The combination of class I and class II alleles, or HLA genotype, personalizes an individual's immune response through three processes. First, given the great polymorphism of the HLA genes in the human population, the peptide repertoire displayed by HLA molecules varies greatly among individuals.⁴ Second, HLA polymorphism affects the T-cell repertoire given that thymocytes undergo selection processes that require interactions between T-cell receptors and peptide-HLA complexes on antigen-presenting cells in the thymus.^{5,6} Third, the development and activity of NK cells are in part controlled by the interaction of KIRs with their cognate HLA class I ligands.^{7,8}

Clinical testing of HLA genes is used for a variety of applications. It contributes to the diagnosis of autoimmune diseases, such as ankylosing spondylitis,⁹ narcolepsy,¹⁰ and celiac disease.¹¹ HLA typing is also used to avoid severe adverse reactions to drugs, such as abacavir, carbamazepine, and allopurinol,¹² and to stratify patients for cancer immunotherapy.¹³ HLA typing is fundamental in matching recipients and donors for solid organ¹⁴ and blood and marrow transplantation.¹⁵ HLA molecules of transfused or transplanted cells and tissues are recognized as foreign by the host's immune system and elicit strong immunologic reactions that can lead to graft rejection and platelet refractoriness.¹⁶ HLA matching at the allele level is critical in hematopoietic cell transplantation. In this setting, HLA

mismatches increase the risk not only of primary graft failure but also of graft-versus-host disease, caused by the immune response of the donor against the recipient and both processes are associated with high morbidity and mortality.

The HLA loci are among the most difficult genes to sequence in the human genome. This is due to sequence homology between the HLA genes, the presence of pseudogenes, as well as the extreme sequence variability of the HLA genes themselves. Because of this complexity and the critical need for accurate results, the development of robust and sensitive HLA typing methods is crucial in the clinical setting.

HLA genes and alleles are named by the World Health Organization Nomenclature Committee of the Factors of HLA system.¹⁷ Each HLA allele name has a unique number corresponding to up to four sets of digits or fields separated by colons. HLA typing can be performed at different levels of resolution depending on the clinical need.¹⁸ Low resolution typing defines the first field or serological equivalent. The main methods are locus-specific PCR followed by hybridization with sequence-specific oligonucleotide (SSO) probes covering polymorphic regions, or PCR using sequence-specific primers (SSPs). A high-resolution typing result is defined as a set of alleles that encode the same protein sequence for the antigen binding site (exons 2 and 3 for class I and exon 2 for class II molecules) and that excludes alleles that are not expressed at the cell-surface. This level of resolution is used for hematopoietic cell transplantation.¹⁹ The main methods are PCR followed by Sanger sequence-based typing (SBT) or by next-generation sequencing (NGS).²⁰ HLA typing results obtained by SBT may be ambiguous because of the lack of sequence phasing and the impracticality of interrogating the whole gene. NGS methods allow phasing of polymorphisms along the length of the whole HLA gene and are high-throughput permitting allele level typing in the clinical setting.²¹ Currently, there are several US Food and Drug Administration (FDA)-approved assays for HLA typing. Many laboratories also use laboratory-developed tests and procedures, which are not FDA approved and must be developed and validated by the laboratory.

Clinical and commercial laboratories that develop new assays need characterized reference materials for test development, test validation, quality control, and proficiency testing. In addition, laboratories that use FDA-approved assays need reference materials to verify assay performance and for quality control. Proficiency testing programs need access to DNA samples with a variety of genotypes to provide comprehensive and up-to-date surveys. Currently, there are only two sources of characterized genomic DNA that are often used as reference materials for HLA testing. The UCLA Immunogenetics Center DNA Reference Panels (UCLA DNA Reference Panels, <http://pathology.ucla.edu/uic-hla-reference-programs>, last accessed January 24, 2018) are of limited supply because they are not derived from cell lines. The International

Histocompatibility Working Group Cell and DNA Bank (Fred Hutch HLA Reference Standards, <https://sharedresources.fredhutch.org/products/hla-reference-standards-ihwg>, last accessed January 24, 2018) houses cell lines, DNA, and cloned HLA genes established in the International Histocompatibility Workshops offer reference panels. However, this collection is not being expanded with new cell lines and is not consistently typed using the current gold standard of NGS. There are no other publicly available sources of reference materials for HLA typing.

The Centers for Disease Control and Prevention (CDC) established the Genetic Testing Reference Materials Coordination Program (GeT-RM) in partnership with the genetics community to improve the availability of genomic DNA reference materials necessary for quality assurance of genetic laboratory testing. To improve the availability of publicly available and renewable, characterized reference materials for HLA typing, the GeT-RM together with three clinical laboratories and the Coriell Cell Repositories, have characterized genomic DNA obtained from a panel of 108 cell lines for all HLA classic polymorphic loci: *HLA-A, B, C, DRB1, DRB3, DRB4, DRB5, DQA1, DQB1, DPA1, and DPB1*. This panel of well-characterized genomic DNA reference materials was developed to support molecular HLA typing, assay development, validation and verification, quality control, and proficiency testing.

Materials and Methods

Cell Line Selection

DNA samples from 106 cell lines from the National Institute of General Medical Sciences Human Genetic Cell Repository at the Coriell Cell Repositories were selected for the study. These samples are ethnically diverse, and all were previously characterized by GeT-RM for five pharmacogenetic loci (*CYP2D6, CYP2C19, CYP2C9, VKORC1, UGT1A1*). GeT-RM also collected data for several other genes, including *HLA-B*, during this study.²² In addition, two cell lines with the *HLA-B*15:02* were established for this project at the Coriell Cell Repositories from residual patient blood. All Coriell DNA and cell line materials are stripped of identifiers on submission and are assigned a Coriell cell line number.

DNA Preparation

Approximately 2 mg of DNA was prepared from each of the selected cell lines by the Coriell Cell Repositories using Genra/Qiagen Autopure (Valencia, CA) as per manufacturer's instructions.

Protocol

Samples that contained 10 µg of DNA from the 108 cell lines were sent to three clinical genetic testing laboratories

that used different technologies for analysis. The laboratories assayed each DNA sample using their standard assay methods as described below. A variety of different methods were used to test the samples to ensure a robust characterization. Consensus genotypes were developed for each of the 11 genes (*HLA-A, B, C, DRB1, DRB3, DRB4, DRB5, DQA1, DQB1, DPA1, and DPB1*) tested by two or three methods for each sample by comparison of the results obtained from each assay. The consensus genotype for each gene in each sample is the result obtained by NGS, which provided the highest level of resolution, and was consistent with the results obtained by the parallel testing using other methods.

HLA Typing Methods

One laboratory characterized each sample for the *HLA-A, B, C, DRB1, DQA1, and DQB1* loci at the intermediate resolution level by PCR-SSO using LABType SSO kits (One Lambda, Canoga Park, CA) and *HLA-A, B, C, DRB1, and DQB1* loci at the high-resolution level by SBT. A second laboratory provided SBT typing for *HLA-DPB1*. SBT was performed using AlleleSEQR HLA-SBT kits (Abbott Molecular, Des Plaines, IL). Allele-specific sequence-based typing was performed as needed to resolve cis-trans ambiguities. The allelic library used was ImMunoGeneTics version 3.13.1. The regions covered were the polymorphic regions of loci *A* and *B* (exons 2, 3, and 4), *C* (exons 2, 3, 4, and 7), *DRB1* (exon 2), *DQB1* (exons 2 and 3), and *DPB1* (exon 2).

A third laboratory tested the same set of samples for *HLA-DPA1* using LABType SSO kits (One Lambda) and *DRB3/4/5* using Olerup SSP kits (Stockholm, Sweden). The same laboratory tested all samples using an NGS assay for the 11 loci. Samples were amplified by long-range PCR using primers that cover from the 5' untranslated region (UTR) to the 3' UTR for *HLA-A, B, C, DQA1, DPA1, and DQB1*. *HLA-DRB1, 3, and 4* primers span from intron 1 to intron 4, and *DRB5* and *DPB1* span from intron 1 to 3' UTR. Amplification and library preparation were performed using HoloType HLA kits (Omixon Inc., Budapest, Hungary) supplemented by in-house primer sets (*HLA-DQA1, DPB1, DPA1, DRB4, and DRB5*) according to the manufacturer's instructions for NGS with the MiSeq system (Illumina, San Diego, CA). The in-house primer sets (*HLA-DQA1, DPB1, DPA1, DRB4, and DRB5*) are now included as part of the HoloType HLA kits. The sequencing strategy included 2 × 250-bp paired-end sequencing with MiSeq 500 cycle version 2 chemistry using full-sized and nano-sized flow cells. DNA sequence data analysis and HLA genotyping were performed using ImMunoGeneTics version 3.28.0 with Omixon Twin version 2.1.2 and GenDx NGSengine analysis software version 2.6.0. The results generated by the two programs were compared using the in-house developed software HLA Inspector version 1.1.

Table 1 HLA-A, B, and C High-Resolution Typing of 108 Genomic DNA Panel

Name	HLA-A	HLA-B	HLA-C
NA02016	*02:01:01	*30:02:01	*53:01:01
NA07439	*29:02:01	*68:02:01	*42:02:01
NA09301	*23:01:01	*30:01:01	*13:02:01
NA10005	*02:01:01	*33:03:01	*15:03:01
NA12244	*02:01:01	*23:01:01	*15:01:01
NA12273	*02:01:01	*32:01:01	*35:01:01
NA16654	*24:02:01	*26:01:01	*15:01:01
NA16688	*24:02:01	*26:01:01	*15:07:01
NA16689	*24:02:01	*30:01:01	*13:02:01
NA17019	*02:01:01	*11:01:01	*15:02:01
NA17020	*11:01:01	*11:01:01	*38:02:01
NA17039	*30:01:01	*31:01:02	*35:08:01
NA17052	*02:01:01	*33:03:01	*39:04
NA17057	*24:20	*33:03:01	*15:11:01
NA17058	*24:02:01	*26:01:01	*13:01:01
NA17073	*02:01:01	*02:01:01	*27:05:02
NA17075	*11:01:01	*32:01:01	*49:01:01
NA17078	*30:04:01	*68:02:01	*44:03:02
NA17084	*11:01:01	*80:01:01	*38:02:02
NA17114	*02:02:01	*11:01:01	*15:17:01
NA17115	*30:02:01	*68:02:01	*15:10:01
NA17119	*03:01:01	*74:01:01	*35:01:01
NA17129	*33:01:01	*74:01:01	*50:01:01
NA17130	*01:01:01	*02:01:01	*08:01:01
NA17201	*01:01:01	*02:01:01	*35:08:01
NA17203	*24:02:01	*26:01:01	*14:01:01
NA17204	*02:01:01	*20:01:01	*39:06:02
NA17205	*03:01:01	*11:01:01	*15:01:01
NA17206	*02:01:01	*03:01:01	*07:02:01
NA17207	*03:01:01	*68:01:02	*35:01:01
NA17208	*01:01:01	*30:01:01	*08:01:01
NA17209	*02:01:01	*26:01:01	*07:02:01
NA17210	*26:01:01	*66:01:01	*38:01:01
NA17211	*24:02:01	*29:02:01	*15:01:01
NA17212	*02:05:01	*11:01:01	*27:02:01
NA17213	*02:01:01	*24:02:01	*27:05:02
NA17214	*01:01:01	*02:05:01	*37:01:01
NA17215	*01:01:01	*02:01:01	*08:01:01
NA17216	*01:01:01	*03:01:01	*08:01:01
NA17217	*02:01:01	*34:02:01	*08:01:01
NA17218	*01:01:01	*01:01:01	*07:02:01
NA17219	*02:01:01	*33:01:01	*07:06:01
NA17220	*24:02:01	*30:02:01	*18:01:01
NA17221	*23:01:01	*24:02:01	*35:02:01
NA17222	*02:01:01	*68:01:02	*40:01:02
NA17224	*02:01:01	*02:01:01	*15:01:01
NA17226	*01:01:01	*34:02:01	*08:01:01
NA17227	*01:01:01	*02:01:01	*08:01:01
NA17228	*01:01:01	*24:02:01	*15:01:01
NA17229	*24:02:01	*31:01:02	*35:01:01
NA17230	*01:01:01	*32:01:01	*15:01:01
NA17231	*03:01:01	*03:01:01	*07:02:01
NA17232	*03:02:01	*33:01:01	*14:02:01
NA17233	*02:01:01	*02:01:01	*35:03:01
NA17234	*02:01:01	*02:01:01	*44:02:01
NA17235	*03:01:01	*31:01:02	*07:02:01
NA17236	*02:01:01	*03:01:01	*07:02:01

(table continues)

Table 1 (continued)

Name	HLA-A		HLA-B		HLA-C	
NA17237	*26:01:01	*30:02:01	*35:01:01	*44:02:01	*04:01:01	*16:04:01
NA17240	*03:01:01	*30:02:01	*07:02:01	*57:01:01	*06:02:01	*07:02:01
NA17242	*02:01:01	*32:01:01	*35:01:01	*40:01:02	*03:04:01	*04:01:01
NA17243	*24:02:01	*29:02:01	*40:01:02	*44:03:01	*03:04:01	*16:01:01
NA17244	*02:01:01	*30:02:01	*13:02:01	*51:01:01	*06:02:01	*14:02:01
NA17245	*02:05:01	*68:02:01	*44:02:01	*53:01:01	*04:01:01	*16:04:01
NA17246	*24:02:01	*25:01:01	*08:01:01	*18:01:01	*07:01:01	*07:01:01
NA17247	*01:01:01	*24:03:01	*44:03:01	*57:01:01	*04:01:01	*06:02:01
NA17248	*02:01:01	*30:01:01	*13:02:01	*38:01:01	*06:02:01	*12:03:01
NA17249	*01:01:01	*32:01:01	*08:01:01	*44:02:01	*05:01:01	*07:01:01
NA17254	*01:01:01	*02:01:01	*08:01:01	*35:01:01	*07:01:01	*15:02:01
NA17256	*01:01:01	*30:01:01	*13:02:01	*40:01:02	*03:04:01	*06:02:01
NA17257	*01:01:01	*11:01:01	*07:02:01	*15:01:01	*03:03:01	*07:02:01
NA17260	*01:01:01	*29:02:01	*08:01:01	*44:03:01	*07:01:01	*16:01:01
NA17261	*01:01:01	*03:01:01	*35:08:01	*44:02:01	*04:01:01	*06:02:01
NA17262	*02:01:01	*11:01:01	*27:05:02	*39:01:01	*02:02:02	*07:02:01
NA17263	*02:01:01	*02:05:01	*44:02:01	*53:01:01	*05:01:01	*06:02:01
NA17264	*01:01:01	*24:02:01	*08:01:01	*35:01:01	*04:01:01	*07:01:01
NA17265	*02:01:01	*26:01:01	*07:02:01	*51:01:01	*07:02:01	*15:02:01
NA17267	*02:01:01	*02:01:01	*15:17:01	*44:02:01	*05:01:01	*07:01:02
NA17268	*01:01:01	*02:01:01	*15:01:01	*52:01:01	*03:03:01	*12:02:02
NA17269	*02:01:01	*26:01:01	*40:01:02	*40:02:01	*02:02:02	*03:04:01
NA17272	*32:01:01	*33:01:01	*14:02:01	*44:02:01	*05:01:01	*08:02:01
NA17274	*02:05:01	*26:01:01	*38:01:01	*58:01:01	*07:18	*12:03:01
NA17275	*02:01:01	*29:02:01	*13:02:01	*44:03:01	*06:02:01	*16:01:01
NA17276	*01:01:01	*29:02:01	*08:01:01	*44:03:01	*07:01:01	*16:01:01
NA17277	*24:02:01	*31:01:02	*27:05:02	*56:01:01	*01:02:01	*02:02:02
NA17279	*01:01:01	*02:01:01	*08:01:01	*39:01:01	*07:01:01	*07:02:01
NA17280	*01:01:01	*11:01:01	*08:01:01	*18:01:01	*07:01:01	*07:01:01
NA17281	*02:01:01	*31:01:02	*39:06:02	*56:01:01	*01:02:01	*07:612
NA17282	*03:01:01	*11:01:01	*35:03:01	*56:01:01	*01:02:01	*04:01:01
NA17283	*02:01:01	*03:01:01	*07:02:01	*07:02:01	*07:02:01	*07:02:01
NA17285	*02:05:01	*23:01:01	*35:02:01	*51:08:01	*04:01:01	*16:02:01
NA17286	*02:01:01	*03:01:01	*14:02:01	*45:01:01	*06:02:01	*08:02:01
NA17287	*03:01:01	*03:02:01	*15:01:01	*35:03:01	*03:03:01	*04:01:01
NA17288	*01:01:01	*02:01:01	*44:02:01	*57:01:01	*05:01:01	*06:02:01
NA17289	*02:01:01	*23:01:01	*14:06:02	*44:02:01	*05:01:01	*08:02:01
NA17290	*11:01:01	*26:01:01	*07:02:01	*57:01:01	*06:02:01	*12:03:01
NA17291	*02:01:01	*02:01:01	*13:02:01	*35:02:01	*04:01:01	*06:02:01
NA17292	*02:01:01	*02:01:01	*07:02:01	*51:01:01	*07:02:01	*14:02:01
NA17293	*02:01:01	*29:02:01	*07:02:01	*44:02:01	*05:01:01	*07:02:01
NA17295	*01:01:01	*24:02:01	*08:01:01	*15:01:01	*03:03:01	*07:01:01
NA17296	*02:236	*24:02:01	*14:02:01	*44:02:01	*05:01:01	*08:02:01
NA17298	*03:01:01	*11:01:01	*07:02:01	*08:01:01	*07:01:01	*07:02:01
NA17300	*03:01:01	*03:01:01	*07:02:01	*53:01:01	*04:01:01	*07:02:01
NA17438	*24:02:01	*68:03:01	*35:01:01	*52:01:02	*03:03:01	*04:01:01
NA17440	*02:01:01	*02:06:01	*15:15	*35:23	*01:02:01	*04:01:01
NA17466	*11:01:01	*23:01:01	*44:03:01	*44:03:01	*04:01:01	*04:01:01
NA17618	*02:01:01	*02:01:01	*15:16:01	*35:12:01	*04:01:01	*14:02:01
NA23090	*11:01:01	*11:01:01	*15:02:01	*51:01:01	*08:01:01	*14:02:01
NA23093	*11:01:01	*11:01:01	*15:02:01	*15:02:01	*08:01:01	*08:01:01

Results

The goal of this study was to generate a comprehensive panel of publicly available, characterized genomic DNA reference materials for testing of 11 *HLA* loci: *HLA-A*, *B*, *C*,

DRB1, *DRB3*, *DRB4*, *DRB5*, *DQA1*, *DQB1*, *DPA1*, and *DPB1*. DNA from a panel of 108 publicly available cell lines derived from donors with a variety of ethnicities was chosen for this study based on the diversity of *HLA B* alleles determined during a previous GeT-RM study.²² It was

Table 2 HLA-DRB1, 3, 4, and 5 High-Resolution Typing of 108 Genomic DNA Panel

Name	HLA-DRB1	HLA-DRB3	HLA-DRB4	HLA-DRB5
NA02016	*08:04:01	*14:54:01	*02:02:01	
NA07439	*04:03:01	*15:03:01		*01:01:01
NA09301	*07:01:01	*11:04:01	*02:02:01	
NA10005	*04:01:01	*15:03:01		*01:01:01
NA12244	*04:01:01	*07:01:01		
NA12273	*11:01:01	*13:05:01	*02:02:01	*02:02:01
NA16654	*08:03:02	*12:02:01	*03:01:03	
NA16688	*04:05:01	*12:01:01G	*01:01:02	*01:03:01
NA16689	*07:01:01	*14:03:01	*01:01:02	*01:03:01
NA17019	*09:01:02	*12:02:01	*03:01:03	*01:03:02
NA17020	*08:03:02	*12:02:01	*03:01:03	
NA17039	*04:03:01	*08:04:01		*01:03:01
NA17052	*04:03:01	*13:02:01	*03:01:01	*01:03:01
NA17057	*09:01:02	*15:01:01		*01:03:02
NA17058	*04:05:01	*12:02:01	*03:01:03	*01:03:01
NA17073	*01:01:01	*11:01:01	*02:02:01	
NA17075	*11:01:01	*15:01:01	*02:02:01	*01:01:01
NA17078	*07:01:01	*12:01:01G	*02:02:01	*01:01:01
NA17084	*11:01:01	*15:02:01G	*02:02:01	*01:01:01
NA17114	*03:01:01	*11:02:01	*02:02:01	*02:02:01
NA17115	*08:04:01	*13:03:01	*01:01:02	
NA17119	*08:04:01	*08:06		
NA17129	*01:02:01	*13:01:01	*01:01:02	
NA17130	*08:01:01	*15:03:01		*01:01:01
NA17201	*10:01:01	*11:04:01	*02:02:01	
NA17203	*07:01:01	*14:54:01	*02:02:01	*01:01:01
NA17204	*03:01:01	*07:01:01	*02:02:01	*01:01:01
NA17205	*07:01:01	*15:02:02		*01:03:01
NA17206	*13:01:01	*15:01:01	*01:01:02	*01:02
NA17207	*01:01:01	*07:01:01		*01:01:01
NA17208	*03:01:01	*07:01:01	*01:01:02	*01:03:01
NA17209	*07:01:01	*15:01:01		*01:01:01
NA17210	*04:02:01	*11:01:01	*02:02:01	*01:03:01
NA17211	*12:01:01G	*15:01:01	*02:02:01	*01:01:01
NA17212	*13:02:01	*16:01:01	*03:01:01	*02:02
NA17213	*01:01:01	*04:04:01		*01:03:02
NA17214	*10:01:01	*15:01:01		*01:01:01
NA17215	*03:01:01	*13:02:01	*01:01:02	*03:01:01
NA17216	*01:01:01	*03:01:01	*01:01:02	
NA17217	*01:03:01	*03:01:01	*01:01:02	
NA17218	*03:01:01	*15:01:01	*01:01:02	*01:01:01
NA17219	*04:05:01	*07:01:01		*01:01:01
NA17220	*03:01:01	*11:04:01	*02:02:01	*02:02:01
NA17221	*11:04:01	*11:04:01	*02:02:01	*02:02:01
NA17222	*13:02:01	*14:54:01	*02:02:01	*03:01:01
NA17224	*04:01:01	*04:01:01		*01:03:01
NA17226	*07:01:01	*13:02:01	*03:01:01	*01:03:01
NA17227	*03:01:01	*13:02:01	*01:01:02	*03:01:01
NA17228	*12:01:01G	*13:01:01	*01:01:02	*02:02:01
NA17229	*04:07:01	*14:06:01	*01:01:02	*01:03:01
NA17230	*01:01:01	*04:04:01		*01:03:01
NA17231	*01:01:01	*13:02:01	*03:01:01	
NA17232	*01:02:01	*07:01:01		*01:03:01:02N
NA17233	*01:02:01	*07:01:01		*01:03:01:02N
NA17234	*03:01:01	*08:01:01	*02:02:01	
NA17235	*04:04:01	*15:01:01		*01:03:01
NA17236	*15:01:01	*15:01:01		*01:01:01

(table continues)

Table 2 (continued)

Name	HLA-DRB1		HLA-DRB3		HLA-DRB4		HLA-DRB5
NA17237	*11:04:01	*13:02:01	*02:02:01	*03:01:01			
NA17240	*07:01:01	*13:01:01	*02:02:01		*01:03:01:02N		
NA17242	*01:01:01	*13:02:01	*03:01:01				
NA17243	*07:01:01	*13:02:01	*03:01:01		*01:01:01		
NA17244	*13:01:01	*15:01:01	*01:01:02				*01:01:01
NA17245	*01:02:01	*11:04:01	*02:02:01				
NA17246	*03:01:01	*13:01:01	*01:01:02	*01:01:02			
NA17247	*01:01:01	*13:05:01	*02:02:01				
NA17248	*07:01:01	*11:04:01	*02:02:01		*01:03:01		
NA17249	*03:01:01	*13:01:01	*01:01:02	*01:01:02			
NA17254	*01:03:01	*03:01:01	*01:01:02				
NA17256	*04:01:01	*07:01:01			*01:03:01	*01:03:01	
NA17257	*07:01:01	*11:01:01	*02:02:01		*01:03:01:02N		
NA17260	*03:01:01	*07:01:01	*01:01:02		*01:01:01		
NA17261	*04:01:01	*16:07			*01:03:01		*02:02
NA17262	*04:04:01	*08:01:01			*01:03:01		
NA17263	*07:01:01	*13:02:01	*03:01:01		*01:03:01		
NA17264	*01:03:01	*04:01:01			*01:03:01		
NA17265	*04:04:01	*11:01:01	*02:02:01		*01:03:01		
NA17267	*01:01:01	*13:02:01	*03:01:01				
NA17268	*07:01:01	*15:02:01G			*01:03:01:02N		*01:02
NA17269	*13:01:01	*13:02:01	*02:02:01	*03:01:01			
NA17272	*01:01:01	*01:02:01					
NA17274	*04:02:01	*04:02:01			*01:03:01	*01:03:01	
NA17275	*07:01:01	*07:01:01			*01:01:01	*01:03:01	
NA17276	*03:01:01	*15:01:01	*01:01:02				*01:01:01
NA17277	*08:01:01	*11:01:01	*02:02:01				
NA17279	*03:01:01	*08:01:01	*01:01:02				
NA17280	*03:01:01	*11:04:01	*01:01:02	*02:02:01			
NA17281	*01:01:01	*04:01:01			*01:03:01		
NA17282	*07:01:01	*13:01:01	*02:02:01		*01:03:01:02N		
NA17283	*15:01:01	*15:01:01					*01:01:01 *01:01:01
NA17285	*11:04:01	*13:03:01	*01:01:02	*02:02:01			
NA17286	*07:01:01	*13:02:01	*03:01:01		*01:01:01		
NA17287	*11:01:01	*11:01:01	*02:02:01	*02:02:01			
NA17288	*03:01:01	*07:01:01	*02:02:01		*01:03:01:02N		
NA17289	*01:02:01	*04:01:01			*01:03:01		
NA17290	*14:54:01	*15:01:01	*02:02:01				*01:01:01
NA17291	*07:01:01	*11:01:01	*02:02:01		*01:03:01		
NA17292	*08:03:02	*11:01:01	*02:02:01				
NA17293	*07:01:01	*15:01:01			*01:01:01		*01:01:01
NA17295	*03:01:01	*15:01:01	*01:01:02				*01:01:01
NA17296	*13:02:01	*15:01:01	*03:01:01				*01:01:01
NA17298	*03:01:01	*15:01:01	*01:01:02				*01:01:01
NA17300	*04:04:01	*07:01:01			*01:01:01	*01:03:01	
NA17438	*04:07:01	*14:06:01	*01:01:02		*01:03:01		
NA17440	*08:02:01	*08:02:01					
NA17466	*07:01:01	*07:01:01			*01:01:01	*01:01:01	
NA17618	*01:02:01	*08:02:01					
NA23090	*04:05:01	*09:01:02			*01:03:01	*01:03:02	
NA23093	*12:02:01	*12:02:01	*03:01:03	*03:01:03			

The *DRB1* gene is present in all haplotypes, and a second *DRB* gene, *DRB3*, *DRB4*, or *DRB5* is present in only some haplotypes.

expected that by selecting a diverse set of samples, most of the common and many of the rare genotypes of each gene would be represented. The consensus HLA genotype for each DNA sample is given in [Tables 1, 2, and 3](#).

HLA typing results using SBT, SSP, SSO, and NGS were 100% concordant. Results were compared between NGS and at least one other method. Because of the technologies used, these typing approaches give different kinds of

Table 3 HLA-DQA1, DQB1, DPA1, and DPB1 Typing of 108 Genomic DNA Panel

Name	HLA-DQA1	HLA-DQB1	HLA-DPA1	HLA-DPB1
NA02016	*01:05:01	*04:01:02	*04:02:01	*05:01:01
NA07439	*01:02:01	*03:01:01	*03:02:01	*06:02:01
NA09301	*02:01:01	*05:05:01	*02:02:01	*03:01:01
NA10005	*01:02:01	*03:03:01	*03:01:01	*06:02:01
NA12244	*02:01:01	*03:01:01	*02:02:01	*03:02:01
NA12273	*05:05:01	*05:05:01	*03:01:01	*03:01:01
NA16654	*01:03:01	*06:01:01	*03:01:01	*06:01:01
NA16688	*03:03:01	*05:05:01	*03:01:01	*04:01:01
NA16689	*02:01:01	*05:07	*02:02:01	*03:01:01
NA17019	*03:02	*06:01:01	*03:01:01	*03:03:02
NA17020	*01:03:01	*06:01:01	*03:01:01	*06:01:01
NA17039	*03:01:01	*05:05:01	*03:01:04	*03:02:01
NA17052	*01:02:01	*03:01:01	*03:02:01	*06:04:01
NA17057	*01:02:01	*03:02	*03:03:02	*06:02:01
NA17058	*03:03:01	*06:01:01	*03:01:01	*04:01:01
NA17073	*01:01:01	*05:05:01	*03:01:01	*05:01:01
NA17075	*01:02:01	*05:05:01	*03:01:01	*06:02:01
NA17078	*01:05:01	*02:01:01	*02:02:01	*05:01:01
NA17084	*01:02:01	*05:05:01	*03:01:01	*05:02:01
NA17114	*05:01:01	*05:05:01	*02:01:01	*03:19:01
NA17115	*05:05:01	*05:05:01	*03:01:01	*03:01:04
NA17119	*01:02:01	*05:05:01	*03:01:04	*06:02:01
NA17129	*01:01:02	*01:03:01	*05:01:01	*06:03:01
NA17130	*01:02:01	*04:01:01	*04:02:01	*06:02:01
NA17201	*01:05:01	*05:05:01	*03:01:01	*05:01:01
NA17203	*01:04:01	*02:01:01	*02:02:01	*05:03:01
NA17204	*02:01:01	*05:01:01	*02:02:01	*02:02:01
NA17205	*01:03:01	*02:01:01	*02:02:01	*06:01:01
NA17206	*01:02:01	*01:03:01	*06:02:01	*06:03:01
NA17207	*01:01:01	*02:01:01	*02:02:01	*05:01:01
NA17208	*02:01:01	*05:01:01	*02:01:01	*02:02:01
NA17209	*01:02:01	*02:01:01	*02:02:01	*06:02:01
NA17210	*03:01:01	*05:05:01	*03:01:01	*03:02:01
NA17211	*01:02:01	*05:05:01	*03:01:01	*06:02:01
NA17212	*01:02:01	*01:02:02	*05:02:01	*06:09:01
NA17213	*01:01:01	*03:01:01	*03:02:01	*05:01:01
NA17214	*01:02:01	*01:05:01	*05:01:01	*06:02:01
NA17215	*01:02:01	*05:01:01	*02:01:01	*06:04:01
NA17216	*01:01:01	*05:01:01	*02:01:01	*05:01:01
NA17217	*01:01:01	*05:01:01	*02:01:01	*05:01:01
NA17218	*01:02:01	*05:01:01	*02:01:01	*06:02:01
NA17219	*02:01:01	*03:03:01	*02:02:01	*03:02:01
NA17220	*05:01:01	*05:05:01	*02:01:01	*03:01:01
NA17221	*05:05:01	*05:05:01	*03:01:01	*03:01:01
NA17222	*01:02:01	*01:04:01	*05:03:01	*06:04:01
NA17224	*03:01:01	*03:01:01	*03:02:01	*03:02:01
NA17226	*01:02:01	*02:01:01	*02:02:01	*06:04:01
NA17227	*01:02:01	*05:01:01	*02:01:01	*06:04:01
NA17228	*01:03:01	*05:05:01	*03:01:01	*06:03:01
NA17229	*03:01:01	*05:03	*03:01:01	*03:02:01
NA17230	*01:01:01	*03:01:01	*03:02:01	*05:01:01
NA17231	*01:01:01	*01:02:01	*05:01:01	*06:04:01
NA17232	*01:01:02	*02:01:01	*03:03:02	*05:01:01
NA17233	*01:01:02	*02:01:01	*03:03:02	*05:01:01
NA17234	*04:02	*05:01:01	*02:01:01	*04:02:01
NA17235	*01:02:01	*03:01:01	*03:02:01	*06:02:01
NA17236	*01:02:01	*01:02:01	*06:02:01	*06:02:01
				*01:03:01
				*01:03:01
				*02:01:02
				*03:01:01
				*04:01:01
				*05:01:01
				*06:02:01
				*07:01:01
				*08:01:01
				*09:01:01
				*10:01:01
				*11:01:01
				*12:01:01
				*13:01:01
				*14:01:01
				*15:01:01
				*16:01:01
				*17:01:01
				*18:01
				*19:01:01
				*20:01:01
				*21:01:01
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				*23:01:01
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				*30:01:01
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				*35:01:01
				*36:01:01
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				*38:01:01
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				*40:01:01
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				*42:01:01
				*43:01:01
				*44:01:01
				*45:01
				*46:01:01
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				*48:01:01
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				*51:01:01
				*52:01:01
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				*54:01:01
				*55:01:01
				*56:01:01
				*57:01:01
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				*60:01:01
				*61:01:01
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				*66:01:01
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				*71:01:01
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				*73:01:01
				*74:01:01
				*75:01:01
				*76:01:01
				*77:01:01
				*78:01:01
				*79:01:01
				*80:01:01
				*81:01:01
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				*83:01:01
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				*85:01:01
				*86:01:01
				*87:01:01
				*88:01:01
				*89:01:01
				*90:01:01
				*91:01:01
				*92:01:01
				*93:01:01
				*94:01:01
				*95:01:01
				*96:01:01
				*97:01:01
				*98:01:01
				*99:01:01
				*100:01:01
				*101:01:01
				*102:01:01
				*103:01:01
				*104:01:01
				*105:01:01
				*106:01:01
				*107:01:01
				*108:01:01

(table continues)

Table 3 (continued)

Name	HLA-DQA1	HLA-DQB1	HLA-DPA1	HLA-DPB1
NA17237	*01:02:01	*05:05:01	*03:01:01	*06:09:01
NA17240	*01:03:01	*02:01:01	*03:03:02	*05:03:01
NA17242	*01:01:01	*01:02:01	*05:01:01	*06:04:01
NA17243	*01:02:01	*02:01:01	*02:02:01	*06:04:01
NA17244	*01:02:01	*01:03:01	*06:02:01	*06:03:01
NA17245	*01:01:02	*05:05:01	*03:01:01	*05:01:01
NA17246	*01:03:01	*05:01:01	*02:01:01	*06:03:01
NA17247	*01:01:01	*05:05:01	*03:01:01	*05:01:01
NA17248	*02:01:01	*05:05:01	*02:02:01	*03:01:01
NA17249	*01:03:01	*05:01:01	*02:01:01	*06:03:01
NA17254	*01:01:01	*05:01:01	*02:01:01	*05:01:01
NA17256	*02:01:01	*03:01:01	*02:02:01	*03:02:01
NA17257	*02:01:01	*05:05:01	*03:01:01	*03:03:02
NA17260	*02:01:01	*05:01:01	*02:01:01	*02:02:01
NA17261	*01:02:02	*03:03:01	*03:01:01	*05:02:01
NA17262	*03:01:01	*04:01:01	*03:02:01	*04:02:01
NA17263	*01:02:01	*02:01:01	*02:02:01	*06:04:01
NA17264	*01:01:01	*03:01:01	*03:02:01	*05:01:01
NA17265	*03:01:01	*05:05:01	*03:01:01	*03:02:01
NA17267	*01:01:01	*01:02:01	*05:01:01	*06:04:01
NA17268	*01:03:01	*02:01:01	*03:03:02	*06:01:01
NA17269	*01:02:01	*01:03:01	*06:03:01	*06:04:01
NA17272	*01:01:01	*01:01:02	*05:01:01	*05:01:01
NA17274	*03:01:01	*03:01:01	*03:02:01	*03:02:01
NA17275	*02:01:01	*02:01:01	*02:02:01	*02:02:01
NA17276	*01:02:01	*05:01:01	*02:01:01	*06:02:01
NA17277	*04:01:01	*05:05:01	*03:01:01	*04:02:01
NA17279	*04:01:01	*05:01:01	*02:01:01	*04:02:01
NA17280	*05:01:01	*05:05:01	*02:01:01	*03:01:01
NA17281	*01:01:01	*03:01:01	*03:02:01	*05:01:01
NA17282	*01:03:01	*02:01:01	*03:03:02	*06:03:01
NA17283	*01:02:01	*01:02:01	*06:02:01	*06:02:01
NA17285	*05:05:01	*05:05:01	*03:01:01	*03:01:01
NA17286	*01:02:01	*02:01:01	*02:02:01	*06:09:01
NA17287	*05:05:01	*05:05:01	*03:01:01	*03:01:01
NA17288	*02:01:01	*05:01:01	*02:01:01	*03:03:02
NA17289	*01:01:02	*03:03:01	*03:01:01	*05:01:01
NA17290	*01:02:02	*01:04:01	*05:02:01	*05:03:01
NA17291	*02:01:01	*05:05:01	*02:02:01	*03:01:01
NA17292	*05:05:01	*06:01:01	*03:01:01	*03:01:01
NA17293	*01:02:01	*02:01:01	*02:02:01	*06:02:01
NA17295	*01:02:01	*05:01:01	*02:01:01	*06:02:01
NA17296	*01:02:01	*01:02:01	*06:02:01	*06:09:01
NA17298	*01:02:01	*05:01:01	*02:01:01	*06:02:01
NA17300	*02:01:01	*03:01:01	*02:02:01	*03:02:01
NA17438	*03:01:01	*05:03	*03:01:01	*03:02:01
NA17440	*04:01:01	*04:01:01	*04:02:01	*04:02:01
NA17466	*02:01:01	*02:01:01	*02:02:01	*02:02:01
NA17618	*01:01:02	*04:01:01	*04:02:01	*05:01:01
NA23090	*03:02	*03:03:01	*03:03:02	*04:01:01
NA23093	*06:01:01	*06:01:01	*03:01:01	*03:01:01

genotyping results. NGS provides a definitive genotype without ambiguities, whereas the other methods are less precise and report a number of different possible allele combinations (ambiguity strings). As such, concordance is

then defined as agreement between the NGS genotype and the presence of the NGS genotype within the ambiguity string provided by the legacy methods. Concordance was determined between methods accounting for typings

Table 4 List of HLA Alleles Found at Least Once in the 108 Genomic DNA Panel

HLA-A	HLA-B	HLA-C	HLA-DRB1	HLA-DRB3	DQA1	DQB1	DPA1	DPB1
*01:01:01	*07:02:01	*01:02:01	*01:01:01	*01:01:02	*01:01:01	*02:01:01	*01:03:01	*01:01:01
*02:01:01	*07:06:01	*02:02:02	*01:02:01	*02:02:01	*01:01:02	*02:02:01	*02:01:01	*01:01:02
*02:02:01	*08:01:01	*02:10:01	*01:03:01	*03:01:01	*01:02:01	*03:01:01	*02:01:02	*02:01:02
*02:05:01	*13:01:01	*03:03:01	*03:01:01	*03:01:03	*01:02:02	*03:01:01	*02:01:08	*02:01:19
*02:06:01	*13:02:01	*03:04:01	*04:01:01		*01:03:01	*03:01:04	*02:02:02	*02:02:01
*02:236	*14:01:01	*03:04:02	*04:02:01	DRB4	*01:04:01	*03:02:01	*02:06	*03:01:01
*03:01:01	*14:02:01	*04:01:01	*04:03:01	*01:01:01	*01:05:01	*03:03:02	*02:10	*04:01:01
*03:02:01	*14:06:02	*05:01:01	*04:04:01	*01:03:01	*02:01:01	*03:19:01	*03:01	*04:02:01
*11:01:01	*15:01:01	*06:02:01	*04:05:01	*01:03:01:02N	*03:01:01	*04:01:01	*03:NEW-1	*05:01:01
*23:01:01	*15:02:01	*07:01:01	*04:07:01	*01:03:02	*03:02	*04:02:01	*03:NEW-2	*06:01:01
*24:02:01	*15:03:01	*07:01:02	*07:01:01		*03:03:01	*05:01:01		*09:01:01
*24:03:01	*15:07:01	*07:02:01	*08:01:01	DRB5	*04:01:01	*05:02:01		*10:01:01
*24:20	*15:10:01	*07:04:01	*08:02:01	*01:01:01	*04:01:02	*05:03:01		*11:01:01
*25:01:01	*15:11:01	*07:06	*08:03:02	*01:02	*04:02	*06:01:01		*13:01:01G
*26:01:01	*15:15	*07:18	*08:04:01	*02:02	*05:01:01	*06:02:01		*14:01:01
*29:02:01	*15:16:01	*07:612	*08:06		*05:03	*06:03:01		*15:01:01
*30:01:01	*15:17:01	*08:01:01	*09:01:02		*05:05:01	*06:04:01		*16:01:01
*30:02:01	*18:01:01	*08:02:01	*10:01:01		*05:07	*06:09:01		*17:01:01
*30:04:01	*27:02:01	*08:03:01	*11:01:01		*06:01:01			*18:01
*31:01:02	*27:05:02	*12:02:02	*11:02:01					*21:01
*32:01:01	*35:01:01	*12:03:01	*11:04:01					*23:01:01
*33:01:01	*35:02:01	*14:02:01	*12:01:01G					*26:01:02
*33:03:01	*35:03:01	*14:03	*12:02:01					*45:01
*34:02:01	*35:08:01	*15:02:01	*13:01:01					*104:01:01
*66:01:01	*35:12:01	*15:05:02	*13:02:01					*105:01:01
*68:01:02	*35:20:01	*16:01:01	*13:03:01					*124:01:NEW
*68:02:01	*35:23	*16:02:01	*13:05:01					*131:01
*68:03:01	*37:01:01	*16:04:01	*14:03:01					*350:01
*74:01:01	*38:01:01	*17:01:01	*14:06:01					*584:01
*80:01:01	*38:02:01	*17:03:01	*14:54:01					
	*38:02:02		*15:01:01					
	*39:01:01		*15:02:01G					
	*39:04		*15:02:02					
	*39:06:02		*15:03:01					
	*40:01:02		*15:02:01G					
	*41:01:01		*15:02:02					
	*42:02:01		*16:01:01					
	*44:02:01		*16:07					
	*44:03:01							
	*45:01:01							
	*49:01:01							
	*50:01:01							
	*51:01:01							
	*51:08:01							
	*52:01:01							
	*53:01:01							
	*55:01:01							
	*56:01:01							
	*57:01:01							
	*57:03:01							
	*58:01:01							

reported at two or three fields, depending on the level reported by the legacy methods. Of the 1905 alleles typed, no discordance was identified.

Five unique novel alleles with differences in exonic regions were identified across a total of six loci, and the

sequences of three of the novel alleles are being submitted to the World Health Organization Nomenclature Committee for naming. The first phased novel allele, *C*07:612* (sample NA17281), differs from *C*07:02:01:01* in exon 5 at codon 277. The codon change from TCT to TTT results in an

amino acid change from serine to phenylalanine (<https://www.ncbi.nlm.nih.gov/genbank>; accession number MG845550). The second phased novel allele, *DPA1*02:10* (sample NA17020), differs from *DPA1*02:02:02* in exon 3 at codon 96. The codon change from CCT to GCT results in an amino acid change from proline to alanine (GenBank accession number MG938526). The third phased novel allele, *DPA1*03:NEW-1* (sample NA17119), is an *HLA-DPA1* allele that differs from *DPA1*03:01* in exon 1 at codon -31, which is the translation start site (GenBank accession number MG836705). The codon change from ATG to ACG (methionine to threonine) is presumed to shift the translation start to the next ATG, which is in frame at codon -25. This would, in theory, create a translated peptide that is six amino acids shorter than the canonical *DPA1*03:01* allele. More work is being performed to determine the effect of this nucleotide change on the RNA and protein expression before the allele is officially named. Besides these three phased and well-characterized sequences, there are three other unique novelties that were observed in exonic sequences but could not be fully phased across the targeted region and therefore will not be submitted to the World Health Organization Nomenclature Committee for naming. Two of the novel alleles are unique and are from samples NA17219 and NA10005. Sample NA17219 has a novel *HLA-DPBI* allele compared with *DPBI*124:01* in exon 5 at codon 225, which changes CAA to CAG and is synonymous for glutamine, *DPBI*124:01:NEW*. Sample NA10005 has a novel *HLA-DPA1* allele compared with *DPA1*03:01* in exon 4 at codon 190, changing the codon ACG to GCG, resulting in an amino acid change from threonine to alanine, *DPA1*03:NEW-2*. Although the novel nucleotides could not be fully phased across the entire amplicon, each nucleotide substitution was able to be phased to other known heterozygous positions that occur between the alleles in the sample and thus assigned to a single allele. A third unphased sequence (sample NA17115) exhibited the same nucleotide change as in sample NA17119 at the *DPA1* locus, whereby the translation start codon -31 is changed from ATG to ACG, *DPA1*03:NEW-1*. In this second sample, NA17115, the combination of alleles is different from that of sample NA17119, whereby the base change in exon 1 could not be phased with exon 2. Therefore, even though it is assumed to be the same novel *DPA1*03* allele, the sequence was not submitted to the World Health Organization Nomenclature Committee.

Ambiguities persisted in 50/1905 allele calls, with most attributable to alternative cis/trans combinations of exons 2 and 3 of *DPBI* ($n = 21$ pairs: 42 alleles) and eight caused by polymorphisms in a noncovered region (exon1) of the *DRB1* and *DPBI* genes. According to official nomenclature, G codes were used to designate a group of HLA alleles that have identical nucleotide sequences across the exons encoding the peptide-binding domains. For *HLA-DRB1*12:01:01G*, *DRB1*15:02:01G*, and *DPBI*13:01:01G*, the alleles

*HLA-DRB1*12:10* (four cases), *DRB1*15:140* (two cases), and *DPBI*107:01* (two cases), respectively, could not be ruled out because of a lack of exon 1 coverage. All other *DPBI* G groups were reported as G groups because exons 2 and 3 could not be phased and an alternative set of alleles existed that covered the known heterozygous positions. In these cases, the distances between heterozygous positions were 700 to 4000 bases apart.

A total of 236 unique alleles were identified. Thirty *HLA-A*, 54 *-B*, 30 *-C*, 36 *-DRB1*, 4 *-DRB3*, 4 *-DRB4*, 3 *-DRB5*, 19 *-DQA1*, 17 *-DQB1*, 10 *-DPA1*, and 29 *-DPBI* alleles in different heterozygous and homozygous combinations are represented in the panel and are listed in Table 4. These alleles cover a high percentage of HLA specificities in each of five different ancestry groups in the US population: European, African American, Asian/Pacific Islander, Hispanic, and Native American (Supplemental Tables S1 and S2). Supplemental Tables S3, S4, and S5 list the alleles at a two-field resolution and their cumulative frequencies in the different populations. For *HLA-A*, *B*, *C*, *DRB1*, 3, 4, 5, and *DQB1* frequencies were obtained from the Be The Match Registry²³ (Be The Match Registry Haplotype Frequencies, <https://bioinformatics.bethematchclinical.org/HLA-Resources/Haplotype-Frequencies/Be-The-Match-Registry-Haplotype-Frequencies>, last accessed November 10, 2017). Allele frequencies for *HLA-DQA1*, *DPA1*, and *DPBI* were obtained from The Allele Frequency Net Database²⁴ (<http://www.allelefrequencies.net>, last accessed November 10, 2017) and US population data complemented with data from countries that represented different ancestry groups.

Discussion

This study describes the characterization of 108 genomic DNA reference materials for HLA genetic testing. The initiative led by CDC, in the context of the GeT-RM program, provides publicly available and renewable characterized reference materials for HLA typing. Each DNA sample has been characterized for all HLA classic polymorphic loci: *HLA-A*, *B*, *C*, *DRB1*, *DRB3*, *DRB4*, *DRB5*, *DQA1*, *DQB1*, *DPA1*, and *DPBI*. This reference material can be used by laboratories and other test developers to comply with regulatory and accreditation requirements^{25,26} (New York State Clinical Laboratory Evaluation Program, <https://www.wadsworth.org/regulatory/clep>, last accessed January 24, 2018) for assay development, assay validation, and verification, as well as quality control and proficiency testing. Many professional guidelines^{27–30} recommend the use of reference materials to ensure the quality of the tests.

Reference materials should be thoroughly characterized using a variety of analytic methods to confirm the presence of the expected variants and polymorphisms. The samples were tested in three clinical laboratories using a variety of DNA sequence analysis methods to ensure a robust and

complete characterization of the 11 genes studied. The characterization of the samples by NGS secured the best-quality typing results possible. No discordant typings were found on comparison of genotypes reported by the legacy methods and that of NGS.

Even though the NGS data may provide information that would enable the reporting of the alleles at the four-field level, it was not included for two reasons. First, based on the study design, the typing at the fourth field (introns) derived through NGS cannot be compared with the results from the legacy methods because these intronic sequences are not characterized by the legacy methods. Second, HLA genotyping at four fields remains a challenge in the laboratory because there are many alleles that are not defined at the fourth field, having no characterized sequence in the intronic and/or UTR regions. In addition, for alleles that are defined at the fourth field, it is not uncommon to find differences in the lengths of homopolymers and other short tandem repeat regions that occur in the introns. It can be difficult to sequence these repeat structures and accurately call the proper number of repeats, which can change the fourth-field interpretation. In the absence of a second laboratory that would confirm our intronic sequences with an independent NGS method, four-field results were not reported. Finally, clinical laboratories that are implementing NGS are typically not using the fourth field information for clinical decision making.

Reference materials should closely resemble patient samples and should contain variants and polymorphisms that are common to the disorder being tested. Overall, the total number of alleles included in the 108 DNA samples for each of the 11 loci cumulatively covered a high percentage of allelic frequencies in five different ancestry groups in the US population: Caucasian, African American, Asian or Pacific Islander, Hispanic, and Native American. Thus, the variety of HLA alleles in this sample set will allow laboratories to have reference materials for most of the HLA alleles commonly found in their testing populations and should allow test developers and users to evaluate the ability of their assays to detect most common HLA variants.

This collection of 108 well-characterized, cell-line–based DNA samples are publicly available from the National Institutes of General Medical Science Repository at the Coriell Cell Repositories (Coriell Cell Repositories, Camden, NJ). More information about the GeT-RM program and other genomic DNA reference materials are available on the GeT-RM website (<https://wwwn.cdc.gov/clia/Resources/GetRM/default.aspx>).

The complete sequence of the novel *C* allele, including exons 1 to 7 and all encompassed introns, and the complete sequence of the novel *DPA1* allele, including exons 1 to 4 and all encompassed introns, were submitted to GenBank (<https://www.ncbi.nlm.nih.gov/genbank>; accession numbers MG845550 and MG938526, respectively) and to the World Health Organization HLA Nomenclature

Committee for factors of the HLA system. The name *C*07:612* and *DPA1*02:10*, respectively, were officially assigned by the World Health Organization Nomenclature Committee in February 2018 following the policy stipulated in the most recent Nomenclature Report.¹⁷

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Supplemental Data

Supplemental material for this article can be found at <https://doi.org/10.1016/j.jmoldx.2018.05.009>.

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