Corporate Medical Policy

Red Blood Cell Molecular Testing AHS-M2170

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Description of Procedure or Service

The first successful blood transfusion can be dated back to the 1600s (Osterman & Arora, 2017), and molecular testing methods to analyze blood samples were introduced to the transfusion medicine community in the 1990s (Westhoff, 2006). Several ailments may warrant a blood transfusion, and the phenotypic and genotypic determination of red blood cell antigens assist in limiting immune responses in transfusion patients. Agglutination tests via serology have been the gold standard for determining blood group antigens for more than 100 years (Boccoz, Le Goff, Blum, & Marquette, 2015), yet newer molecular techniques may grant added specificity (Elite, 2015a).

Related Policies:
Prenatal Screening AHS-G2035
Genetic Testing for Alpha- and Beta Thalassemia AHS-M2131

******Note: This Medical Policy is complex and technical. For questions concerning the technical language and/or specific clinical indications for its use, please consult your physician.***

Policy

BCBSNC will provide coverage for red blood cell molecular testing when it is determined to be medically necessary because the medical criteria and guidelines shown below are met.

Benefits Application

This medical policy relates only to the services or supplies described herein. Please refer to the Member's Benefit Booklet for availability of benefits. Member's benefits may vary according to benefit design; therefore member benefit language should be reviewed before applying the terms of this medical policy.

When Red Blood Cell Molecular testing is covered

Red cell genotyping (including C, c, D, E, e, K, k, Jka, Jkb, Fya, Fyb, S, s,U) is considered **medically necessary** for the following:

a. Individuals with sickle cell disease, thalassemia syndromes, hemoglobinopathies, and/or other medical conditions requiring recurring transfusions since phenotyping may be misleading due to the presence of transfused cells

b. Individuals with post-transfusion hemolysis when no antibodies are detectable, and no other possible cause is known (e.g. sickle cell crisis or mechanical hemolysis due to heart valve failure)

c. Individuals who received a transfusion in the previous three months AND who anticipate additional transfusions

d. Individuals with autoimmune hemolytic anemia

e. In multiply transfused individuals and/or direct antiglobulin test positive (DAT+) patients

f. Pregnant individuals with non-transfusion-dependent thalassemia (NTDT) prior to transfusion

g. To aid in management of hemolytic disease of the fetus and newborn (HDFN)

h. To resolve conflicting serological antibody results

Please refer to AHS policy G2035 "Prenatal Screening" for guidance regarding fetal RHD genotyping using maternal plasma in RHD negative pregnant women.

When Red Blood Cell Molecular testing is not covered

Red cell genotyping (including C, c, D, E, e, K, k, Jka, Jkb, Fya, Fyb, S, s, U) is considered **investigational** for the following:

a. For individuals who have had allogenic hematopoietic stem cell transplants

b. For diagnosis of sickle cell disease

c. For routine pre-transfusion testing

d. For routine solid organ transplant patients screening

Note: For 5 or more gene tests being run on the same platform, such as multi-gene panel next generation sequencing, please refer to AHS-R2162 Reimbursement Policy.

Policy Guidelines

Transfusion medicine requires extensive blood type knowledge to ensure a safe and efficient transfusion process. Blood type differs throughout the population based on an individual's genome, and genes can vary due to allelic variations. Alleles are alternative forms of the same gene that arise by mutation. Alleles cause genomic changes that ultimately lead to a discrepancy in the proteins produced in the body. Single-nucleotide polymorphisms (SNPs) are also found in the genome and describe a location where the nucleotide that is present differs between individuals because a substitution has been made. Alleles are sometimes caused by SNPs but can also be caused by other genetic mutations as well, such as additions, deletions, and insertions. Further, SNPs do not always lead to new alleles, as they could occur in non-coding areas of the genome. These alleles and SNPs play an important role in blood type and, therefore, in blood transfusion outcomes. Genetic variations will always affect an organism's genotype, which is the set of genes carried by the individual's cells, but may or may not affect an organism's phenotype, which are the observable characteristics or physical expression of those genes.

Blood Groups

Blood is quite complex and can be categorized into several different groups. Historically, blood groups have been classified by the antigens found "on or in the surface of erythrocytes which are present in certain individuals of a species but are lacking in others (Kabat, 1956)." These antigens are proteins with the ability to promote an immune response in the body by inducing the formation of antibodies.

To date, dozens of blood groups have been identified. As of April 2021, the International Society of Blood Transfusion has recognized 43 blood group systems (ISBT, 2020). This number seems to be constantly changing as new discoveries are made. Only a few years earlier, McBean, Hyland, and Flower (2014) reported that 35 blood group systems had been identified, as well as 44 blood group genes and 1568 alleles. Most of these blood group antigens result from SNPs (Boccoz et al., 2015).

Karl Landsteiner, an Austrian biologist in the twentieth century, received the Nobel Prize for the discovery of the first human blood grouping system, ABO, in the early 1900s (Harmening & Firestone, 2012). The ABO group was later classified into four antigens (A, B, O and AB) and six genotypes (AA, AO, BB, BO, OO and AB) in 1924 (Zhang et al., 2015). Examples of these are shown below in Table 1. As likely inferred from the name, the ABO blood group has three main allelic forms at its chromosomal locus: A, B and O. An individual's ABO blood type is genetically inherited, and A and B alleles are inherited in a codominant manner over O (Harmening & Firestone, 2012). The ABO locus has been identified on chromosome 9 at 9q34.1-q34.2; differences in the A and B alleles have been identified and include variations in seven nucleotides via substitution, with four translating into different amino acids (Bethesda, 2005).

Another inherited antigen found on the surface of red blood cells is the Rhesus (Rh) antigen (+/-); after the ABO blood group, the Rh factor is the most clinically significant factor (Písačka, 2018). The Rh blood group system, also identified by Karl Landsteiner alongside Alexander Weiner, was named for the rhesus monkey from which this blood group was originally discovered (Agre, Smith, & Hartel-Schenk, 1990). The Rh+ genotype is completely dominant over Rh-. It was recently reported that the Rh blood group system is comprised of 54 antigens and is "the most complex and polymorphic system" (Raud et al., 2017). Several journal articles have identified genetic variants in the RH (*RHD* and *RHCE*) genes, including SNP substitutions, insertions, deletions, and rearrangements; however, the expression of these variants require further research to determine how overall blood type is affected (Raud et al., 2017). Westhoff (2006) states that more than 100 different *RHD* genes have been identified which encode "proteins with single amino acid changes, or rearranged genes encoding hybrid proteins, and over 50 different *RHCE* genes with single or multiple amino acid changes." Genetic variation in the *RHD* and *RHCE* genes, including partial D antigens, are common, especially in individuals with African descent; further, the weak D phenotype is commonly identified in Caucasians (Elite, 2015b). Other minor blood group systems include Kell, Kidd, Duffy, MNS, P, Lewis, Junior, and Lutheran (Kahar & Patel, 2014; Raud et al., 2017).

Blood Transfusions

Certain antibodies are considered more clinically significant than others for blood transfusion purposes. Clinically significant antibodies (with antigens listed in the subsequent parentheses) include "ABO (A, B), Rh (D, C, c, E, e), Duffy (Fya, Fyb), Kidd (Jka, Jkb), Kell (K, k), and SsU (S, s, U)" and less clinically significant antibodies include "Lewis (Le^a, Le^b), MN, P1, Xg^a, Cartwright (Yt^a), Bg, Knops (Kn^a, McC^a, Yk^a), Chido/Rodgers (Ch1/Rg1), Sda; as well as high titer low avidity (HTLA) antibodies" (Uhl, 2021).

The ABO blood group is widely accepted as the most important of all blood systems for blood transfusion and transplant purposes. This is because the ABO group is responsible for most immune responses due to blood type incompatibility (Hu, Zhang, & Zeng, 2019). Further, ABO is "the only blood group system in which individuals predictably have antibodies in their serum to antigens" absent from their red blood cells (Harmening & Firestone, 2012). It is known that individuals with type A blood (containing A antigens) have anti-B antibodies in their serum, individuals with type B blood (containing B antigens) have anti-A antibodies in their serum, individuals with type O blood (containing no antigens) have anti-A and anti-B antibodies in their serum, and individuals with type AB blood (containing A and B antigens) do not have any anti-A or anti-B antibodies in their serum (Bethesda, 2005). ABO antibodies form in the serum naturally and are stimulated when the immune system encounters the "missing" ABO blood group antigen from food or in micro-organisms; "This happens at an early age because sugars that are identical to, or very similar to, the ABO blood group antigens are found throughout nature (Bethesda, 2005)." Although every red blood cell reportedly expresses

about 2 million ABO blood group antigens, ABO antigens are not only present on red blood cells; these antigens are also found on many types of human tissue and most endothelial and epithelial cells (Bethesda, 2005). In certain individuals, these antigens can also be found in saliva and most bodily fluids as well (Bethesda, 2005).

Table 1: Phenotypes, Genotypes and Antibodies of the ABO Blood Group

Blood Type	Phenotype (i.e. antigen)	Potential Genotype(s)	Antibodies found in serum
A	A	AA, AO	Anti-B
B	B	BB, BO	Anti-A
AB	A and B	AB	None
O	Neither A nor B	OO	Anti-A and Anti-B

Due to the presence of antigens and antibodies on red blood cells, safe blood transfusions rely heavily on blood type knowledge. Administration of the wrong blood type can immediately lead to red blood cell lysis and death, and it is a leading cause of transfusion-related death as reported to the Food and Drug Administration (FDA) (Harmening & Firestone, 2012). If the incorrect blood type is given to a patient, antibodies begin complement causing "rapid intravascular hemolysis, triggering an acute hemolytic transfusion reaction that can cause disseminated intravascular coagulation, shock, acute renal failure, and death" (Bethesda, 2005).

Several medical procedures utilize blood antigen typing such as blood transfusions, bone marrow transplants, stem cell transplants, and organ transplants. Blood transfusions may be administered for a multitude of reasons, including severe blood loss, leukemia, sickle cell disease, thalassemia, anemia, and kidney disease. Bone marrow, which is found in several adult bones including those of the pelvis, can assist with the transplant of healthy blood stem cells to replace others that have been destroyed by disease or infection; bone marrow transplants are known to assist in the treatment of several cancers, including leukemia, myeloma and lymphoma (Miller et al., 2019; G. L. Shah et al., 2018). Several factors—including age, illness severity, co-morbidities, and hemorrhage amount and rate—should be considered before a blood transfusion occurs (Osterman & Arora, 2017; Yaddanapudi & Yaddanapudi, 2014). Still, it has been reported that the use of blood transfusions for medical practice has been notoriously overused since the 20th century for the treatment of anemia and hemorrhage (Yaddanapudi & Yaddanapudi, 2014). Based on data distributed by the Red Cross, more than 30 million blood components are transfused annually in the United States (Osterman & Arora, 2017). In addition to whole blood transfusions, several types of blood product transfusions are available, including platelet, plasma and cryoprecipitate (Yaddanapudi & Yaddanapudi, 2014).

Hematopoietic Stem Cell Transplantation (HSCT)

Hematopoietic stem cells are immature cells with the ability to develop into all types of blood cells including red blood cells, white blood cells, and platelets. The transplantation of these cells, known as a hematopoietic stem cell transplantation (HSCT), is a medical procedure which involves the intravenous transfusion of hematopoietic stem cells obtained from the same (autologous) or different (allogenic) individual. These stem cells can be obtained from the umbilical cord, bone marrow, or peripheral blood and may be used to improve hematopoietic function or fight diseases such as leukemias, lymphoid neoplasia, solid tumors and non-malignant disorders (Passweg et al., 2016).

In contrast to the requirement of ABO match for other types of blood transfusions and solid organ transplants, ABO mismatch is acceptable in HSCTs (Shokrgozar & Tamaddon, 2018). HSCTs can be safely matched by the human leukocyte antigen (HLA), which is inherited independently of the ABO

antigens (Booth, Gehrie, Bolan, & Savani, 2013; Shokrgozar & Tamaddon, 2018). Approximately 40-50% of all HSCTs will involve recipient-donor ABO incompatibility (Booth et al., 2013; Worel, 2016). However, an ABO mismatch may cause the recipient to experience pure red blood cell aplasia, delayed hemolytic reactions, or delayed red blood cell engraftment (Booth et al., 2013). Researchers note that outcomes in ABO-incompatible HSCT have yielded inconsistent results, and pre-transplant antigen evaluation may be important to determine the extent of the incompatibility (Booth et al., 2013; Parkhideh et al., 2019).

Solid Organ Transplants

Solid Organ transplants are common between blood group- compatible individuals, with this process currently considered the most effective treatment for end-stage organ failure (Hu et al., 2019). To date, most important tissues in the body have been successfully transplanted, with exception of the brain.

Solid organ transplant is generally completed with ABO identical donor organs; however, minor ABO-incompatible solid organ transplants may be performed (Uhl, 2021). While ABO compatibility is widely accepted as the primary immunologic barrier for solid organ transplants, human leukocyte antigen (HLA) compatibility is also very salient (Fasano et al., 2017). The HLA complex is a key part of the immune system controlled by genes on chromosome six; the HLA loci are part of the major histocompatibility complex (MHC) which helps the immune system to recognize foreign molecules (Shankarkumar, Ghosh, & Mohanty, 2002). Some HLA antigens are recognized on all tissues in the body (as opposed to mainly red blood cells), making these antigens an integral part of organ and tissue transplants. The importance of HLA antibodies has been studied extensively in renal transplants "where recipient antibodies directed against mismatched donor HLA antigens are associated with antibody mediated rejection and/or graft loss" (Fasano et al., 2017).

Hemoglobinopathies (Sickle Cell Disease and Thalassemia)

Hemoglobinopathies, such as sickle cell disease and thalassemia, are red blood cell disorders. Sickle cell disease is a collection of inherited blood disorders which cause the development of abnormal sickle-shaped red blood cells that disrupt blood flow and lead to a plethora of problems, including ischemia and inflammation (Ware, de Montalembert, Tshilolo, & Abboud, 2017). Approximately 300,000 new blood disorder cases are reported globally each year due to sickle cell disease (Strouse, 2016). Blood transfusions for those with sickle cell disease can assist with an increase in oxygenation to tissues in the body, restoration of blood volume, reduction of sickle erythropoiesis, and prevention of acute vaso-occlusion (Ware et al., 2017). Alloimmunization, which is when the immune system responds to foreign antigens after a blood transfusion, occurs in a percentage of transfusion cases. "Extended red blood cell (RBC) antigen matching is recommended to limit alloimmunization in patients with sickle cell disease (Casas et al., 2015)." Thalassemia is an inherited blood disorder in which an abnormal or inadequate amount of hemoglobin is produced. Thalassemia can be categorized as α-or β-thalassemia depending on which hemoglobin chain is affected (Brancaleoni, Di Pierro, Motta, & Cappellini, 2016). Blood transfusions are a potential treatment option for individuals with this disease (Dhawan et al., 2014).

However, post-transfusion complications are frequent with hemoglobinopathies due to "subtle antigenic differences between donors and patients" (Belsito, Magnussen, & Napoli, 2017). For sickle cell disease, red blood cell alloimmunization is common, and red blood cell antigen discrepancies are particularly important to identify because delayed transfusion reactions may be life threatening (Ware et al., 2017). Regarding thalassemia, alloimmunization rates emphasize "the need for RBC antigen typing before first transfusion and issue of antigen matched blood (at least for Rh and Kell antigen) (Dhawan et al., 2014)." Patients with sickle cell disease and thalassemia develop alloantibodies at a much higher rate than typical transfusions; patients with diseases such as these, or other patients who receive blood transfusions often, will benefit from an extended phenotype determination either with serological methods or genotypic methods (Elite, 2015b). Fasano and Chou (2016) have used red blood cell antigen genotyping for sickle cell disease, thalassemia and other complications that may arise from transfusions; "Genotyping can be used to determine RBC antigen phenotypes in patients recently

transfused or with interfering allo- or autoantibodies, to resolve discrepant serologic typing, and/or when typing antisera are not readily available (Fasano & Chou, 2016)."

Blood Transfusion Molecular Testing

For more than 100 years, agglutination tests using serology have been considered the gold standard for determining blood group antigens (Boccoz et al., 2015). These agglutination tests are simple to perform and identify antibodies or antigens in a sample, allowing for blood type to be inferred. Antiglobulin (Coombs) testing is a common laboratory technique that utilizes a secondary antibody to detect antibodies in the patient's serum; direct antiglobulin testing detects antibodies on the surface of red blood cells, and indirect antiglobulin testing identifies alloantibodies in patient serum (Uhl, 2021). However, these methods have limitations, such as inadequate immunological reagent availability, rising reagent costs, and specificity discrepancies between minor and rare blood types (Boccoz et al., 2015; Westhoff, 2006). Further, a recent red blood cell transfusion may also affect serologic results.

Certain immunohematological tests, such as adsorption/elution techniques, enzyme tests and indirect antiglobulin tests, are important for the detection of specific weak D phenotypes, but other weak D phenotypes cannot be detected by serological techniques (Guzijan, Jovanovic Srzentic, Pavlovic Jankovic, Djilas, & Lilic, 2019). Weak or partial blood type antigens such as Rh, Fy^b, Jk^a, and U have been known to cause blood typing discrepancies (Elite, 2015b). Moreover, certain blood type antigens exist for which "there is no commercially available antisera and poorly characterized patient-source antisera (eg., hrB, hrS, Joa, Hy, U)" (Elite, 2015b). These limitations are significant because researchers have noted that "In practice, serological matching for ABO blood group antigens, Rhesus antigens (RhD, RhC, and RhE), and Kell (K) antigens does not fully prevent alloimmunization, because of RH alleles with reduced or altered antigen expression (Ware et al., 2017)."

To combat serologic discrepancies, several DNA-based molecular testing methods may now be used for blood transfusion purposes. These methods can resolve the red blood cell inconsistencies often caused by weak and partial antigens (Belsito et al., 2017). Nonetheless, molecular typing is expensive, and results may take a few days or weeks to be returned. Further, for the majority of transfusion recipients, matching beyond ABO and D blood type antigens is not necessary (Anstee, 2009). It is, therefore, unlikely that these methods will become a substitute for serological testing but may be used instead when more detailed results are required (Sapatnekar, 2015).

These molecular techniques include Sanger sequencing of both genomic DNA and complimentary DNA as well as SNP assay testing; an SNP assay may consist of polymerase chain reaction (PCR)-restriction fragment length polymorphism (PCR-RFLP) or sequence-specific PCR (SSP-PCR) (Elite, 2015b). Additional DNA amplification techniques may also be used for red blood cell genotyping, including "PCR-single-strand conformation polymorphism, allele-specific-PCR, PCR-amplified product length polymorphism, reverse transcription-quantitative (RT-q)PCR and DNA chip (Zhang et al., 2015)." Further, next generation sequencing is considered very promising for HLA typing, but more research is required to determine if this method is useful in the identification of blood group antigens (Elite, 2015b). Other methods include array-based techniques which allow for multi-parallel testing where numerous samples can be searched at once for SNPs or other analytes (Elite, 2015b). Microarrays, which may be planar arrays or suspended bead arrays, help to overcome several serology limitations by "replacing the immunochemical reagents by synthetic and controlled probes" (Boccoz et al., 2015).

Genotyping assays from whole-blood samples, which use an on-chip test to identify up to 24 blood group antigens, are also available (Boccoz et al., 2015), and next generation sequencing (NGS) methods can detect *de novo* genetic polymorphisms, SNPs, and other genetic variants (Belsito et al., 2017). Liu, Liu, Mercado, Illoh, and Davey (2014) claim that NGS methods "assess genetic variations that cannot be achieved by traditional Sanger sequencing or other genotyping platforms." More recently, it has been reported that molecular diagnostics for red blood cell genotypes can now be determined in the

clinical laboratory and that the majority of allelic variations can be contributed to SNPs (Wilkinson, 2016).

A few panel tests have been developed that identify several blood antigens simultaneously. The BLOODchip® ID CORE XT™ by Grifols Diagnostic Solutions Inc. is an FDA-approved test. The BLOODchip® ID CORE XT™ can simultaneously identify 37 blood antigens in the following blood group systems: Rh, Kell, Kidd, Duffy, MNS, Diego, Dombrock, Colton, Cartwright and Lutheran (Grifols, 2018). This kit will be particularly beneficial to patients who require ongoing blood transfusions, such as individuals with sickle cell disease and thalassemia, as well as for cancer patients who may require very thorough blood typing. This kit uses PCR to amplify DNA samples and can provide results in approximately four hours. A study of 1000 samples reported a sensitivity of 100% and, after bidirectional sequencing, a 100% specificity for the BLOODchip® ID CORE XT blood typing assay; one discrepancy was identified in the E- antigen and 33 discrepancies were identified in the Fy^b- antigen (Lopez et al., 2018).

The PreciseType® HEA Test by Immuncor, Inc. is an FDA-approved multiplexed molecular assay which provides information on 35 red blood cell antigens from eleven different blood groups (Rh, Kell, Duffy, Kidd, MNS, Lutheran, Dombrock, Landsteiner-Wiener, Diego, Colton and Scianna) (Immuncor, 2020). This test can also identify 24 gene mutations and one SNP commonly associated with hemoglobinopathies. This test uses PCR and requires no confirmation of results with antisera (Immuncor, 2020).

There are many other assays not yet FDA-approved but are used for research and investigational purposes only. The chart below displays various blood group genotyping methodologies which detect polymorphisms and antigens (Sippert, 2019).

Name/Manufacturer	**Principle**	**Number of polymorphisms and antigens identified**
Immucor PreciseType Molecular BeadChip by BioArray Solutions (FDA approved)	Multiplex PCR followed by hybridization of amplified DNA to probes attached to spectrally distinguishable beads and elongation	24 polymorphisms associated with 38 antigens plus phenotypic variants and Hemoglobin S
BioArray RHCE and RHD BeadChip by BioArray Solutions	Multiplex PCR followed by hybridization of amplified DNA to probes attached to spectrally distinguishable beads and elongation	35+ *RHCE* variants; 80+ *RHD* variants
ID CORE XT by Progenika Biopharma (FDA-approved)	Multiplex biotinylating PCR followed by hybridization to probes on color-coded microspheres	29 polymorphisms associated with 37 antigens and phenotypic variants
ID RHD XT by Progenika Biopharma	Multiplex biotinylating PCR followed by hybridization to probes coupled to color-coded microspheres	6 *RHD* variants and HPA-1
Hemo ID DQS Panel by Agena Bioscience	Multiplex end-point PCR and single base primer extension	101 antigens associated with 16 systems, and 23 platelet and neutrophil antigens, in modules
HIFI Blood 96 by AXO Science	Automated multiplex PCR and microarray-based assay	15 polymorphisms associated with 24 antigens

RBC-FluoGene vERYfy eXtend by inno-train diagnostik GMBH	Automated TaqMan-based assay	70 antigens and phenotypic variants associated with 12 systems
RBC-ReadyGene by inno-train diagnostic GMBH	PCR-SSP-based assay	16 modules encompassing antigens and variant phenotypes within 13 systems
TaqMan genotyping on OpenArray by BioTrove	Nanofluidic TaqMan assays performed in OpenArray plates	16, 32 or 64 assays (custom designed)
Pre-designed TaqMan assays Thermo Fisher Scientific	Real-time allelic discrimination PCR	Polymorphisms associated with 8 systems

Analytical Validity

A novel method for ABO genotyping was developed by Muro et al. (2012) which used allele-specific primers and real-time PCR; this method can be completed in less than two hours, detects SNPs at three different nucleotide positions in the ABO gene, and can determine six main ABO genotypes. The authors state that "this method could be applicable for rapid and simple screening of forensic samples (Muro et al., 2012)." However, this method is limited for transfusion medicinal purposes as it only identifies ABO genotypes.

Zhang et al. (2015) developed a method of determining ABO blood group genotypes with a real-time loop-mediated isothermal amplification method (LAMP); this LAMP method used two SNPs at nucleotides 261 and 803 to differentiate six ABO genotypes in less than an hour. A total of 101 samples were tested in this study, and each sample resulted in an ABO genotype; "The results were compared with the phenotypes determined by serological assay and the genotypes determined by direct sequencing, and no discrepancies were observed (Zhang et al., 2015)." This shows that this LAMP method is an accurate and efficient method to assist in ABO blood type genotyping.

More recently, Sillence et al. (2017) used digital PCR to analyze samples taken from 53 donors, and compared these results with traditional serological data. The researchers stated that "results showed clear and reliable determination of RHD zygosity using digital PCR." Four discrepancies were identified between genotypic and serological results. All results were checked by other methods, including sanger sequencing, long-range PCR and next-generation sequencing, revealing that the digital PCR method was correct every time (Sillence et al., 2017). This digital PCR method provides a rapid and accurate response regarding Rh phenotype and genotypes and will assist in transfusion medicine molecular testing, paternal and fetal RH zygosity identification, and in hemolytic disease of the fetus and newborn cases.

Krog, Rieneck, Clausen, Steffensen, and Dziegiel (2019) have developed 35 PCR assays for blood donor genotyping. This high-throughput PCR genotyping method analyzed samples from 1034 patients, identifying 62 discrepancies between initial results, 43 of which are due to serology techniques (Krog et al., 2019). The accuracy of the genotyping method was determined at 99.9% with only 17 errors; "Of 17 discrepancies caused by the genotype, three were incorrect antigen-negative predictions and could potentially, as the solitary analysis, have caused an adverse transfusion reaction (Krog et al., 2019)." This is an extremely sensitive and cost-efficient method with a high capacity for screening many donors.

Another set of researchers developed a method of amplifying DNA via PCR "directly from plasma or serum of blood donors followed by a melting curve analysis in a capillary rapid-cycle PCR assay" (Wagner, Flegel, Bittner, & Doscher, 2017). This method allows the DNA extraction step to be skipped, permitting this assay to be completed in only 40 minutes. A positive predictive value was identified at 100%, and a negative predictive value at 84%. The authors state that "With fast turnaround times, the

rapid-cycle PCR assay may eventually be developed and applied to red cell genotyping in the hospital setting (Wagner et al., 2017)."

It has been reported that the genetic Rh complexity among sickle cell disease patients is so great that molecular assays are not detailed enough to identify all possible genetic variants; therefore, next generation sequencing has been employed to identify all Rh genetic variants in an effort to improve transfusion outcomes in sickle cell disease patients (Dezan et al., 2017). This study used samples from 35 sickle cell disease patients with "unexplained Rh antibodies"; a genotypic method which used *RHD* and *RHCE* gene-specific primers was compared to traditional serological data. It was reported that "Ten and 25 variant *RHD* and *RHCE* alleles were identified, respectively. Among all cases of unexplained Rh antibodies, 62% had been inaccurately classified by serological analysis and, of these, 73.1% were considered as relevant (Dezan et al., 2017)." This next generation sequencing assay is therefore an effective measure to identify genetic variants and improve blood transfusion outcomes in the sickle cell disease population.

Molecular *RHD* typing was implemented at two institutions in Europe via PCR-SSP; this was completed to assist in blood transfusion cases (Guzijan et al., 2019). This molecular method confirmed the majority of serologically identified weak D variants with more detail, and it also identified a discrepancy in one sample (Guzijan et al., 2019). This study has verified the accuracy of this genotyping method to confirm *RHD* variants, which will assist in the implementation of safer blood transfusion practices.

A. Shah et al. (2019) compared serological and molecular detection methods for common red cell antigen groups in thalassemia major and sickle cell disease patients. Specifically, the authors compared the molecular genotyping of Rh (D, C, c, E, e), Kell (K, k), Duffy (Fy^a, Fy^b) and Kidd (Jk^a, Jk^b) blood group antigens using PCR and PCR-RFLP to red blood cell antigen phenotypes identified by serology; serological testing was completed by tube agglutination. The authors note that the serological testing for extended RHCcEe, Kell, Kidd and Duffy testing for multi-transfused patients may not generate the correct blood grouping of the recipient. The authors report that "In more than 80% of the cases recipient's molecular testing blood groups were at variance with serologically tested blood groups ($p < 0.0001$). Mixed field reactions in serological typing were common. In sickle cell anemia patients, no discrepancy was found (A. Shah et al., 2019)." This shows that molecular detection methods to determine the genotype was particularly useful in multi-transfused thalassemia patients.

Sheppard et al., (2020) investigated the diagnostic accuracy of two PCR-based group genotyping platforms for patients with sickle cell disease (SCD) including the human erythrocyte antigen (HEA) BeadChip (Immucor, Norcross, GA) and ID CORE XT (Progenika-Grifols, Barcelona, Spain). DNA samples were extracted from 138 patients with SCD to compare predicted phenotype results and calculate a concordance rate. As a result, predicted phenotype results were comparable across platforms for 31 antigens and the concordance rate was 99.9%. The study also identified discrepancies between the two assays in the highly variable Rh blood group system. In some cases, the two assays interrogated different positions in the gene, and the software made different assumptions based on the population frequency of the alleles. The ID CORE XT was able to identify the phenotype in 2 of 3 samples which had discrepancies in the Rh blood group while the HEA BeadChip identified the phenotype in 1 of 3 samples due to lack of interrogation in the gene. The authors conclude that genotyping provides valuable information not available by serologic methods and both methods are beneficial due to the high concordance rate (Sheppard et al., 2020).

Clinical Validity and Utility

It is imperative that patients with sickle cell anemia receive correct red blood cell transfusions. In an effort to compare the accuracy of new DNA-based testing methods to traditional agglutination methods, Casas et al. (2015) compared 494 red blood cell phenotypes of sickle cell disease patients determined by hemagglutination methods to genotypic predictions made by DNA-based testing methods. If any discrepancies were identified, repeat serologic testing was performed to confirm these results. It was found that "Seventy-one typing discrepancies were identified among 6360 antigen comparisons (1.1%).

New specimens for repeat serologic testing were obtained for 66 discrepancies and retyping agreed with the genotype in 64 cases (Casas et al., 2015).” This shows that DNA-based red blood cell genotyping can provide more accurate results when compared to traditional serologic methods, leading to more safe and efficient treatments for sickle cell disease patients.

Flegel, Gottschall, and Denomme (2015) completed a large-scale blood genotyping project; samples were analyzed from 43,066 blood donors from the Blood Center of Wisconsin. Red blood cell genotyping was completed with “a nanofluidic microarray system, using 32 single nucleotide polymorphisms to predict 42 blood group antigens” (Flegel et al., 2015). This microarray technique allowed researchers to fill 95% of antigen-negative red blood cell requests for hospitals served by the Blood Center of Wisconsin; the remining requests were filled via serology identification. This genotyping system is therefore efficient and should be used along with conventional serology methods.

The frequency of red blood cell autoimmunization and alloimmunization development in β-thalassemia major patients was studied by Dhawan et al. (2014); clinical and transfusion records from 319 patients were analyzed. A total of 18 patients were found to have alloantibodies, 90 patients developed autoantibodies, and 9 patients had both alloantibodies and autoantibodies; “Alloimmunization was detected in 5.64% of multi-transfused thalassemia patients. Rh and Kell blood group system antibodies accounted for more than 80% of alloantibodies. This study re-emphasizes the need for RBC antigen typing before first transfusion and issue of antigen matched blood (at least for Rh and Kell antigen) (Dhawan et al., 2014).”

Parkhideh et al. (2019) studied the effects of ABO incompatibility on the outcome of allogenic HSCTs. This retrospective study includes data from 186 different patients with 108 identical, 38 minor, 32 major and 8 bidirectional ABO incompatible scenarios. The researchers did not identify any significant associations between ABO incompatibility and white blood cell or platelet engraftment, or graft-versus-host disease; however, white blood cell engraftment was significantly lower in patients with minor ABO incompatibilities (Parkhideh et al., 2019).

Shih et al. (2020) discussed using red cell antigen genotyping and serological phenotyping in SCD patients to risk-stratify patients for alloimmunization risk. In this study, 106 SCD patients underwent genotyping to assess whether partial antigen expression may lead to increased risk of alloimmunization and hemolytic transfusion reactions (HTRs). 91% of the 106 patients showed additional clinically relevant genotyping information when compared to serological phenotyping alone. The FY*02N.01 was found in 90% of patients and RH variant alleles were found in 49% of patients, with most of them having putative partial antigen expression. Overall, genotyping helped discover more clinically relevant information and "genotyping SCD patients before transfusion may prevent alloimmunization and HTRs." Additionally, "knowledge of the FY*02N.01 variant allele increases feasibility of finding compatible blood (Shih et al., 2020)."

Guidelines and Recommendations

The AABB and The Molecular Testing Standards Committee (MT SC) (MTSC, 2014)

The 2nd edition of the MT SC for red cell, platelet and neutrophil antigen guidelines states that “When a serological reagent is not available, molecular results shall be confirmed by another method (e.g., DNA sequencing) or an external laboratory (MTSC, 2014).” This committee is spearheaded by the AABB (formerly known as the American Association of Blood Banks).

American Society of Hematology (ASH) (Chou et al., 2020)

The ASH has published guidelines for sickle cell disease transfusion support. These guidelines give the following recommendations:

- "The ASH guideline panel suggests an extended red cell antigen profile by genotype or serology over only ABO/RhD typing for all patients with SCD (all genotypes) at the earliest opportunity (optimally before the first transfusion) (conditional recommendation based on very low certainty in the evidence about effects).
- The ASH guideline panel recommends prophylactic red cell antigen matching for Rh (C, E or C/c, E/e) and K antigens over only ABO/RhD matching for patients with SCD (all genotypes) receiving transfusions (strong recommendation based on moderate certainty in the evidence about effects (Chou et al., 2020)."

British Committee for Standards in Hematology (BCSH) (Milkins et al., 2013) updated 2017

The BCSH has published guidelines on pre-transfusion compatibility procedures in blood transfusion laboratories. These guidelines state the following:

"If a patient shows signs of active haemolysis following transfusion but no antibodies are detectable and no other possible cause is known (e.g. sickle cell crisis or mechanical haemolysis due to heart valve failure), this can be due to very rare examples of antibodies which are not detectable by normal serological techniques. In this case:

i. Serological investigation should be undertaken using more sensitive techniques such as Polyethylene Glycol or enzyme IAT, which may require referral to a reference center.
ii. It may be appropriate to fully red cell genotype the patient and select donor blood matched as closely as possible.
iii. Advice should be sought from a clinical haematologist or from a reference laboratory at the local Blood Centre (Milkins et al., 2013)."

Regarding antibody screening, the BCSH states that "Antibody screening should always be performed as part of pre-transfusion testing as it provides the laboratory with a more reliable and sensitive method of detecting a red cell antibody than serological crossmatching (Milkins et al., 2013)."

For patients who have received a transfusion in the previous three months, the BCSH recommends:

i. "Phenotyping may be misleading due to the presence of transfused cells; In this case genotyping can be used as an alternative and is available from specialist laboratories.
ii. When an Rh antibody is suspected, the C, c, E, e types should be determined to aid in the selection of appropriately phenotyped red cells.
iii. Where antibody exclusion is problematic, more extensive phenotyping may be informative to identify antigens to which the patient may be alloimmunised (Milkins et al., 2013)."

For patients with autoimmune hemolytic anemia, BCSH guidelines state that the "Risk of transfusion reaction due to underlying alloantibodies can further be reduced by matching blood with the patient's own type. Determination of the phenotype in multiply transfused and/or DAT+ patients may be problematic and determination of genotype offers useful information in managing these complex cases; this particularly applies to regularly transfused cases and those with autoantibodies resistant to removal by adsorption (Milkins et al., 2013)."

Finally, for patients with sickle cell disease, the BCSH recommends the following:

"The patient's red cells should be phenotyped as fully as possible prior to transfusion. Where patients have already been transfused, the genotype can be determined.

i. An extended phenotype (or genotype) should include C, c, E, e, K, k, Jka, Jkb, Fya, Fyb, S, s.
ii. If S- s-, then U typing should be performed.
iii. As a minimum, red cells should be matched for Rh and K antigens (Milkins et al., 2013)."

British Committee for Standards in Hematology (BCSH) (Davis et al., 2017)

The BCSH also published guidelines on red blood cell transfusion in sickle cell disease. The following recommendations are given for investigating a hemolytic transfusion reaction:

"Serological testing on pre-transfusion and post-transfusion blood samples.

- Repeat ABO/Rh D typing
- Check antibody screen on both samples
- Red cell units transfused within 12–24 h should be crossmatched against both the pre- and post-reaction samples
- Check the direct antiglobulin test (DAT). A positive DAT may be encountered as part of an investigation

Selection of red cell units for further transfusion

- Select ABO extended Rh and K matched units negative for the relevant antigen(s) to which there are current or historical antibodies
- Undertake serological crossmatch to check compatibility; electronic issue should not be used
- If the identity of the new alloantibody is in doubt despite further specialist testing, consider providing extended antigen matched blood (if serological phenotyping cannot be used because of the presence of transfused donor red blood cells, the sample should be sent to an appropriate reference laboratory for molecular red cell genotyping) (Davis et al., 2017)."

British Committee for Standards in Hematology (BCSH) (White et al., 2016)

The BCSH also published guidelines for blood grouping and red blood cell testing during pregnancy. These guidelines state that "If potentially clinically significant maternal antibodies have been identified, paternal testing should be considered to predict the risk to current and future pregnancies. This may be particularly relevant if non-invasive fetal genotyping is not available for the corresponding red cell antigen (Grade 1B) (White et al., 2016)."

These guidelines later stated that "Fetal RHD typing using a high-throughput methodology in pregnant women who have not formed anti-D, as part of a screening program to target anti-D Ig prophylaxis, is sufficiently accurate for implementation from 11 weeks gestation. The cost effectiveness of such testing is dependent on provision at a point in the antenatal care pathway that does not necessitate an additional visit (Grade 1C) (White et al., 2016)."

Regarding serological methods, the guidelines state that "Samples from pregnant women with immune anti-D or anti-c should be assessed serologically at 4 weekly intervals to 28 weeks gestation and at fortnightly intervals thereafter until delivery. Such cases should be referred to a fetal medicine specialist

if the antibody reaches the critical level and/or the level is rising significantly, where assessment of the need for further monitoring will be made (Grade 1B)" and "Pregnant women with anti-K or other Kell system antibodies (unless the father is confirmed to be negative for the corresponding antigen) should be assessed serologically at monthly intervals to 28 weeks gestation and at fortnightly intervals thereafter until delivery and referred to a fetal medicine specialist when the antibody is first identified (Grade 1B) (White et al., 2016)."

European Directorate for the Quality of Medicines & HealthCare (EDQM) (EDQM, 2018)

The EDQM has published a guide for the preparation, use and quality assurance of blood components. Regarding blood group testing for donor blood, it is stated that "Each donation should be tested for ABO and RhD blood groups and at least all first-time donors should be tested for clinically-significant irregular red-cell antibodies" and that "ABO and RhD blood groups should be verified on each subsequent donation (EDQM, 2018)." The guidelines also give laboratory testing steps for both serological and NAT testing.

In 2020, the 20th edition of the guide for the preparation, use and quality assurance of blood components was released. EDQM recommends that serological testing is the standard procedure used in most laboratories and molecular testing should be used as a supplemental technique to serological testing. Molecular testing should be performed when the results of serological testing of blood donors and patients are unclear, when there is a suspicion of weak antigens or variants, or when serological reagents directed to specific antigens do not exist or are not readily available. "It can also be useful in multi-transfused patients to assist with the selection of phenotyped red cell components. Testing can be undertaken on samples from blood, amniocentesis, biopsy of chorionic villi, and plasma (EDQM, 2020)."

International Collaboration for Transfusion Medicine (ICTM) (Compernolle et al., 2018)

The ICTM published red blood cell related guidelines for patients with hemoglobinopathies. These background of these guidelines state that "No international consensus exists for antigen matching in patients with SCD and b-thalassemia. Some transfusion medicine services provide preventive phenotype (or genotype) extensive matching for C, c, E, e, and K antigens in addition to routine ABO and D. Additional extended matching for Jka, Jkb, Fya, Fyb, and S, s is offered at some centers. In contrast, others provide ABO and D matched RBCs and switch to more extensive matching only if alloantibodies are detected (Compernolle et al., 2018)."

The following recommendations were given by the ICTM:

- "Patients with SCD who do not have alloantibodies and who are anticipated to have a transfusion (simple or exchange transfusion) should probably be transfused with CcEe and K-matched RBCs to reduce the risk of alloimmunization (low quality of evidence, weak recommendation). RBCs matched for CcEe and K can be provided by phenotyping or genotyping RBCs. The use of phenotyping or genotyping will depend on the costs of each method in each jurisdiction. Genotyping appears to be more accurate.
- Patients with thalassemia syndromes who do not have alloantibodies and who require RBC transfusion should probably be transfused with CcEe and K-matched RBCs to reduce the risk of alloimmunization (low quality of evidence, weak recommendation). RBCs matched for CcEe and K can be provided by phenotyping or genotyping RBCs. The use of phenotyping or

genotyping will depend on the costs of each method in each jurisdiction. Genotyping appears to be more accurate (Compernolle et al., 2018)."

In 2020, the British Journal of Hematology released a position paper reviewing ICTMG guidelines and agreed with the above recommendations (Trompeter, Massey, Robinson, & Committee, 2020).

National Heart, Lung, and Blood Institute (NHLBI) (NHLBI, 2014)

An expert panel from the NHLBI state that "RBC units that are to be transfused to individuals with SCD should include matching for C, E, and K antigens (NHLBI, 2014)." The laboratory method to perform the antigen matching is not mentioned in these guidelines.

Thalassemia International Federation (TIF) (Cappellini, Cohen, Porter, Taher, & Viprakasit, 2014; Taher, Musallam, & Cappellini, 2018)

The TIF published guidelines for the management of non-transfusion dependent thalassemia (NTDT) (Taher et al., 2018). These guidelines recommend that "Pregnant women with NTDT who were previously never- or minimally-transfused, should be considered at high risk of alloimmunization if blood transfusions are to be administered during pregnancy. If blood transfusion is deemed necessary, extended genotype and antibody screening should be performed before giving any transfusion and fully-phenotyped matched blood should be given (Taher et al., 2018)."

The TIF also published guidelines for the management of transfusion dependent thalassemia (TDT) (Cappellini et al., 2014). These guidelines recommend the following for compatibility testing:

- "Before embarking on transfusion therapy, patients should have extended red cell antigen typing that includes at least C, c, D, E, e, and Kell, (though preferably a full red cell phenotype/genotype panel) in order to help identify and characterize antibodies in case of later immunization.
- If the patient is already transfused, antigen typing can be performed using molecular rather than serologic testing.
- All patients with thalassaemia should be transfused with ABO and Rh (C, c, D, E, e) and Kell compatible blood in order to avoid alloimmunization against these antigens."

European Renal Best Practice (ERBP) (Abramowicz et al., 2015)

The ERBP has published guidelines on kidney donor and recipient evaluation and perioperative care. Red blood cell typing methodology is not mentioned in these guidelines.

European Association of Urology (EAU) (Rodriguez Faba et al., 2018)

The EAU published guidelines on renal transplation in 2018. These guidelines include the following strong recommendations:

- "Determine the ABO blood group and the human leukocyte antigen A, B, C and DR phenotypes for all candidates awaiting kidney transplantation.
- Test both the donor and recipient for human leukocyte antigen DQ. Human leukocyte antigen DP testing may be performed for sensitised patients.
- Perform thorough testing for HLA antibodies before transplantation

- Perform adequate cross-match tests to avoid hyper-acute rejection, before each kidney and combined kidney/pancreas transplantation
- Prevent hyper-acute rejection by adequate ABO blood group and HLA matching of donor and recipients (Rodriguez Faba et al., 2018)."

The article also states that "compatibility for ABO blood group antigens and HLA antigens was of critical importance in kidney transplantation. This may change in the future, e.g. in the new U.S. allocation system A2 and A2B donors are transplanted into B recipients. To avoid an increasing imbalance between demand and supply in deceased-donor kidney transplantation in O recipients, ABO identity is demanded by several organ allocation organisations with a few exceptions, e.g. as in zero HLA-A+B+DR-mismatch kidneys. With the introduction of antibody elimination methods, potent immunosuppression and novel agents (e.g. anti B-cell drugs), successful ABO-incompatible living donor transplantations, with good long-term outcomes are possible (Rodriguez Faba et al., 2018)." ABO genotyping is not mentioned in these guidelines.

Kidney Disease Improving Global Outcomes (KDIGO) (Mandelbrot et al., 2020)

The KDIGO published guidelines on the evaluation and care of living kidney donors. The following recommendations were given:

- "Donor ABO blood typing should be performed twice before donation to reduce the risk of unintended blood type incompatible transplantation.
- Human leukocyte antigen (HLA) typing for major histocompatibility complex (MHC) Class I (A, B, C) and Class II (DP, DQ, DR) should be performed in donor candidates and their intended recipients, and donor-specific anti-HLA antibodies should be assessed in intended recipients
- Donor candidates who are ABO blood group or HLA incompatible with their intended recipient should be informed of availability, risks, and benefits of treatment options, including kidney paired donation and incompatibility management strategies (Mandelbrot et al., 2020)."

The guidelines also mention in a rationale section that "ABO blood typing should be performed in living donor candidates before donation, including routine duplicate testing, to reduce the risk of unintended blood type-incompatible transplantation. Unintended ABO-incompatible (ABOi) transplantation should be avoidable with ABO typing of the donor and the recipient; however, human errors have led to cases of accidental ABOi organ transplantation in contemporary practice. ABO-subtype testing should be performed when donation is planned to recipients with anti-A antibodies (Mandelbrot et al., 2020)."

The authors later state that "While recipient care is out of the scope of this guideline, it is important to emphasize that recipient candidates should undergo antidonor antibody examinations, including complement-dependent cytotoxicity or flow cytometry crossmatching and Luminex (Bio-Rad Laboratories, Inc., Hercules, CA) assays to determine the history of sensitization, and this testing should be current before proceeding with donor nephrectomy and living donor transplantation (Mandelbrot et al., 2020)."

Eurotransplant Manual (Heidt, 2015)

Version 3.1 (Chapter 10) of the Eurotransplant Manual states that "In case of stem cell transplantation before organ transplantation, a new HLA typing and AB0 blood group typing should be performed." The specific methodology is not mentioned.

National Marrow Donor Program (NMDP) and Center for International Blood and Marrow Transplant Research (CIBMTR) (Dehn et al., 2019)

The NMDP and CIBMTR have published guidelines on unreleated donors and cord blood units for HSCTs. These guidelines state that "Sufficient high-resolution donor-recipient HLA match is of primary importance in transplantation with adult unrelated donors"; further, "While HLA matching remains the primary criteria for donor selection, non-HLA factors are often considered when selecting donors, including cytomegalovirus serostatus, sex, age, ABO compatibility, prior pregnancies, and larger body weight (Dehn et al., 2019)."

State and Federal Regulations, as applicable

A search of the FDA database on 02/26/2020 using the term "genotyping" yielded 24 results. However, many of these results are not relevant to the transfusion medicine field; pertinent tests are listed below. Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

The Immucor PreciseType Human Erythrocyte Antigen Molecular BeadChip Test

This test was approved by the FDA in 2014 and is an in vitro diagnostic test that is able to molecularly determine allele variants which assist in the prediction of erythrocyte antigen phenotypes (FDA, 2014). This test is known as the first FDA approved device for molecular typing of red blood cells.

ID CORE XT by Progenika Biopharma

This molecular based assay tests for non-ABO red blood cell types. This is a PCR hybridization-based genotyping test and can identify alleles which encode for human erythrocyte antigens in DNA and was approved by the FDA in 2018 (FDA, 2018).

Billing/Coding/Physician Documentation Information

This policy may apply to the following codes. Inclusion of a code in this section does not guarantee that it will be reimbursed. For further information on reimbursement guidelines, please see Administrative Policies on the Blue Cross Blue Shield of North Carolina web site at www.bcbsnc.com. They are listed in the Category Search on the Medical Policy search page.

Applicable service codes: 0001U, 0084U, 0180U, 0181U, 0182U, 0183U, 0184U, 0185U, 0186U, 0187U, 0188U, 0189U, 0190U, 0191UI, 0192U, 0193U, 0194U, 0195U, 0196U, 0197U, 0198U, 0199U, 0200U, 0201U, 0221U, 0222U, 0246U, 81403, 81479

BCBSNC may request medical records for determination of medical necessity. When medical records are requested, letters of support and/or explanation are often useful, but are not sufficient documentation unless all specific information needed to make a medical necessity determination is included.

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Specialty Matched Consultant Advisory Panel 8/2021

Policy Implementation/Update Information

7/28/20 New policy developed. BCBSNC will provide coverage for red blood cell molecular testing when it is determined that the medical criteria and guidelines are met. Medical Director review 7/2020. Policy noticed 7/28/20 for effective date 10/1/20. (lpr)

9/7/21 Specialty Matched Consultant Advisory Panel review 8/18/2021. References updated. No change to policy statement. (lpr)

10/1/21 Reviewed by Avalon 2nd Quarter 2021 CAB. Medical Director review 8/2021. Added PLA codes 0221U, 0222U, 0246U to Billing/Coding section. Updated policy guidelines and references. (lpr)