FDA Executive Summary

Classification of Human Leukocyte, Neutrophil and Platelet Antigen or Antibody Tests

Prepared for the
November 30, 2017
Device Classification Panel Meeting of the
Blood Products Advisory Committee

Table of Contents

1. Introduction

The Food and Drug Administration (FDA) is convening the Blood Products Advisory Committee (BPAC) as a Device Classification Panel (the Panel) to discuss the classification of Human Leukocyte Antigen (HLA) Devices, Human Platelet Antigen (HPA) Devices, and Human Neutrophil Antigen (HNA) Devices. Table 1 shows the device groups for panel discussion.

Table 1: HLA, HPA and HNA Devices

Name	Type of Device
HLA Devices	• Devices that perform HLA Typing • Devices that detect antibodies to HLA
HPA Devices	• Devices that perform HPA Typing • Devices that detect antibodies to HPA
HNA Devices	• Devices that perform HNA Typing • Devices that detect antibodies to HNA

The devices mentioned above meet the legal definition of a medical device provided in section 201(h) of the Federal Food, Drug, and Cosmetic Act (FD&C Act).
HLA, HPA and HNA devices are unclassified devices. The Panel will be asked to provide recommendations for the classification of HLA, HPA and HNA Devices as Class I, Class II or Class III medical devices based on the level of control necessary to provide a reasonable assurance of their safety and effectiveness.

If the Panel believes that classification into Class II is appropriate for the devices mentioned above, the Panel will also be asked to discuss Special Controls that would be necessary to mitigate the risks to health. Special Controls are regulatory requirements for class II devices. They are usually device specific and may include performance standards, postmarket surveillance, special labeling requirements, premarket data requirements, or other controls.

FDA proposes to classify HLA, HPA, and HNA devices as a group of devices (Human Leukocyte, Neutrophil and Platelet antigen or antibody tests), with each device type having its own product code. The rationale to classify these devices together is based on the similarities in the biological properties of the three antigen systems, the use of similar technologies for the detection of antigens and antibodies, the clinical use of the test results, and the Special Controls required to mitigate risks. FDA believes that these are devices that do not differ significantly in purpose, design, materials, energy source, function, or other features related to safety and effectiveness, and for which similar regulatory controls are sufficient to provide reasonable assurance of safety and effectiveness.

This device classification does not address HLA, HPA or HNA in-vitro diagnostic (IVD) assays that are intended for clinical use and designed, manufactured and used within a single laboratory.

1.1. Current Regulatory Pathway for HLA, HPA and HNA Devices

The Federal Food, Drug, and Cosmetic Act (FD&C Act), section 513, established the risk-based device classification system for medical devices. HLA, HPA and HNA devices have not been classified under this statutory provision. Currently these devices are regulated as unclassified devices, subject to 510(k) premarket notification requirements.

The 510(k) premarket notification is a submission made to FDA to demonstrate that the device to be marketed is at least as safe and effective as (i.e., substantially equivalent to) a legally marketed Class I or II device of that same generic type. When determined to be substantially equivalent, the subject device may be legally marketed in the United States (U.S.).

The legally marketed device to which substantial equivalence (SE) is determined is known as the predicate device. A predicate device can be a preamendments device (legally marketed prior to the May 28, 1976 Medical Device Amendments to the FD&C Act) or a postamendments device (legally marketed in the U.S. after the May 28, 1976 Medical Device Amendments to the FD&C Act). Substantial equivalence is based on a comparative assessment with respect to: intended use, design, energy used or delivered, materials, performance, safety, effectiveness, labeling, biocompatibility, standards, and other applicable characteristics that would demonstrate the device is as safe and effective as the predicate device.

1.2. Background on Device Classification

Role of the Device Classification Panel

The purpose of the Device Classification Panel is for the Panel to discuss and recommend the most appropriate classification for HLA, HPA, and HNA devices as Class I, II, or III devices.

Section 513(a) of the FD&C Act (21 U.S.C. 360c (a)) establishes three classes of devices based on the regulatory controls needed to provide reasonable assurance of their safety and effectiveness: class I (general controls), class II (special controls in addition to general controls), and class III (premarket approval in addition to general controls).

Pursuant to section 513(d) of the FD&C Act (21 U.S.C. 360c (d)), FDA promulgates classification regulations classifying devices by generic type. A generic type of deviceis a grouping of devices that do not differ significantly in purpose, design, materials, energy source, function, or any other feature related to safety and effectiveness, and for which similar regulatory controls are sufficient to provide reasonable assurance of safety and

effectiveness (21 CFR 860.3(i)). FDA has issued regulations classifying the majority of preamendments devices by generic type of device (See 21 CFR 860.84). Each classification regulation, located in 21 CFR parts 862-892, indicates the class (I, II, or III) in which FDA has classified the device type.

Following a classification recommendation by the Panel, FDA will propose a regulation that identifies and classifies HLA, HPA and HNA devices as generic device types. Once finalized, this regulation will be published in the Code of Federal Regulations (CFR).

Device Regulatory Controls

All classes of medical devices are subject to General Controls. General Controls are the basic provisions (authorities) of the May 28, 1976 Medical Device Amendments to the FD&C Act, that provide the FDA with the means of regulating devices to ensure their safety and effectiveness. They include provisions that relate to adulteration; misbranding; device registration and listing; premarket notification; banned devices; notification including repair, replacement, or refund; records and reports; restricted devices; and good manufacturing practices.

Class I devices are subject to General Controls and are typically exempt from submission of a premarket 510(k) notification. They generally pose the lowest risk to the patient and/or user. Examples of Class I devices include enema kits and elastic bandages.

Class II devices are subject to Special Controls in addition to General Controls. Special Controls may include compliance with a recognized standard, warnings statements in the instructions for use, specific performance requirements, and/or other controls necessary to ensure a reasonable assurance of safety and effectiveness. Class II devices typically require FDA clearance of a premarket 510(k) notification to permit the device to be marketed and sold in the U.S. Examples of Class II devices are cystic fibrosis DNA Assays and Factor VIII antibody screen assays.

Class III devices tend to present higher risk and they may be first-of-a-kind devices where General and Special Controls are not adequate to ensure their safety and effectiveness. Class III devices require FDA approval in the form of a premarket approval (PMA) application prior to marketing in addition to compliance with device General Controls. Examples of Class III devices are DNA-based blood typing assays and HIV drug resistance phenotyping assays.

Device classification

FDA is proceeding through the following steps in order to classify HLA, HPA, and HNA devices per FD&C Act 513:

STEP 1:
FDA collects and reviews available information to identify valid scientific evidence relevant to the safety and effectiveness of HLA, HPA, and HNA devices and weighs the probable benefit of these devices in accordance with their intended use against the probable risks.

STEP 2:
FDA convenes a public meeting of the BPAC's Device Classification Panel to request the Panel's overall assessment of valid scientific evidence pertaining to the probable benefit versus the probable risks of HLA, HPA, and HNA devices, and to obtain the Panel's recommendation on the classification. The Panel meeting provides an opportunity for members of the public, including medical device manufacturers, to present information to the panel during the public hearing portion of the meeting.

The Panel's recommendation for a proposed class for HLA, HPA, and HNA devices considers the regulatory controls necessary to provide reasonable assurance of the safety and effectiveness of these devices for their intended use.

STEP 3:
After receipt of a classification recommendation from the Panel, FDA publishes a proposed classification regulation in the Federal Register and provides interested persons an opportunity to submit comments on the proposed regulation.

STEP 4:
FDA reviews and considers all comments submitted by the public on the proposed regulation.

STEP 5:
After review and consideration of all comments, FDA publishes a final regulation in the Federal Register classifying HLA, HPA, and HNA devices into Class I, II or III. The class, and any Special Controls if applicable, will establish which regulatory controls apply to this device type.

1.3. Device Descriptions

1.3.1. HLA Devices

There are two categories of HLA devices: 1) HLA typing devices: for determination of the HLA type (phenotype or genotype); 2) HLA antibody detection devices: for screening or identifying antibodies to HLA antigens. The device may consist of a single reagent, a test kit with all the necessary reagents, or be designed as a system consisting of reagents, instruments and software. Software used to support data calculation or generate final results may be

submitted to the FDA as part of a system, or in a separate premarket submission. Since HLA devices are currently unclassified, there is no codified FDA regulation associated with the HLA devices.

1.3.1.1. HLA Typing Devices

The HLA genotyping assays often use the following techniques: polymerase chain reaction sequence-specific primer (PCR-SSP), sequence-specific oligonucleotide probe (SSOP), and sequence-based techniques (SBT) including the recently developed next generation sequencing assays. Early versions of HLA typing devices were serological assays. Most laboratories currently use nucleic acid-based assays as the HLA typing method of choice [1].

HLA typing techniques for devices cleared by FDA are detailed below.

Serological HLA typing

- Serological HLA typing: Commonly employs the complement dependent microlymphocytotoxic technique. This method uses characterized HLA antibodies to specific HLA antigens. The HLA antibodies are incubated with viable lymphocytes and complement. If the HLA antigen expressed on lymphocytes is recognized by a specific antibody, a complement dependent microlymphocytotoxic reaction will be triggered leading to cell lysis.

Nucleic acid-based typing

- PCR-SSP: HLA alleles have a characteristic patchwork of single-nucleotide polymorphisms (SNPs). In PCR-SSP, the primers are designed based on these SNPs so that different primers are ideally suited to define different HLA alleles [1]. Amplification patterns are recorded using an agarose gel documentation system and interpreted, usually by software, to obtain HLA types. The HLA system is highly polymorphic, includes a huge number of alleles, and more alleles are being identified. Therefore, the manufacturer of related devices may need to update the primer set and/or the HLA allele assignments for some primers.
- SSOP: The SSOP technique also utilizes the patchwork of SNPs characteristic of HLA. PCR is used to amplify the HLA gene and the denatured amplicons are mixed with probes, which are oligonucleotides specific for different HLA alleles. Specific probe binding indicates the presence of certain HLA alleles. Many HLA SSOP kits utilize bead array technology on the Luminex® platform [1]. Reaction patterns are usually interpreted by software. If new alleles are identified, the manufacturer of related devices may need to update the probe set and/or the HLA allele assignments.
- SBT: DNA sequencing, such as the Sanger method of dideoxy chain termination, has been developed for allelic HLA typing. The Sanger sequencing method utilizes locus- or group-specific amplification, followed by sequencing by capillary electrophoresis. Sequence data are analysed and interpreted by software dedicated to HLA typing. The primers in the sequencing assays may not need to be updated as frequently as the primers or probes used in the SSP or SSOP assays.

1.3.1.2. HLA Antibody Detection Devices

Early versions of HLA antibody detection assays were cell-based and used the complement-dependent cytotoxicity (CDC) technique. In recent years, solid phase assays have been introduced for HLA antibody detection. These assays fall into two major categories: Enzyme-linked immunosorbent assay (ELISA)-based methods and bead-based assays.

- CDC assays: Test serum is incubated with cells of known HLA types, followed by the addition of complement and the use of cell dyes to assess the degree of cell death. Variations to this technique have been developed to improve sensitivity, such as the use of anti-human globulin as a second antibody and the use of extended incubation times. The same technique is used to perform cross-matching of transplant recipients test serum against potential donor cells.
- ELISA: Purified HLA proteins are bound to the surface of plastic wells. Test serum is added to the wells and antibodies, if present, bind to the HLA antigens of appropriate specificity. A second enzyme-linked anti-human immunoglobulin antibody is added followed by a suitable substrate, with a color change indicating a positive reaction.
- Bead-based assays: This method employs many groups of beads that are spectrally distinguishable. Each fluorescently-encoded bead is coated with an HLA antigen or antigens. The test serum is incubated with the bead mix and HLA antibodies present in the test serum bind to the antigens on the beads. The beads are then washed and labeled with a secondary fluorochrome-conjugated antibody. A Luminex® instrument or a conventional flow cytometry analyzer simultaneously detects the signal from the secondary antibody and from the signature dyes of each bead group [2]. The reaction patterns (positive vs. negative reactions) and HLA antigen specificity can then be determined, usually by software.

1.3.2. HPA devices

There are two categories of HPA devices: 1) HPA typing devices: for use in determining the HPA type (phenotype or genotype); 2) HPA antibody detection devices: for screening or identifying antibodies to HPA antigens. The devices may consist of all the necessary reagents in a kit, or be designed as a system consisting of reagents, instrument and software. Software used to support the evaluation and interpretation of HPA results may be submitted to the FDA as part of a system, or in a separate premarket submission. Since HPA devices are unclassified, there is no codified regulation associated with the HPA devices.

1.3.2.1. Human Platelet Antigens Typing Devices

Historically, serologic HPA typing was performed using characterized typing sera containing antibodies to specific HPA antigens, and free of HLA class I antibodies. With the development of PCR and the knowledge of the underlying genetic variation in the human genome of all antigens of the HPA system, serological platelet antigen typing has

been largely replaced by genotyping methods [3].The genotyping methods that are now available to predict human platelet antigens are similar to the methods used for HLA typing devices, including PCR-SSP, SSOP, and SBT. (See section 1.3.1.1)

FDA has cleared devices that use some of the technologies described above. The information about FDA-cleared devices is included in Appendix B, Literature Review.

1.3.2.2. Human Platelet Antibodies Detection Devices

Human platelet antibodies are commonly detected with assays that use intact platelets or antigen-capture methodologies. Examples of such assays are described below.

Assays Using Intact Platelets

- Flow Cytometry: Washed platelets are incubated with patient serum. The platelet-bound antibodies are detected with a fluorescent-labeled (usually fluorescein isothiocyanate—FITC) polyclonal or monoclonal antibody specific for human immunoglobulin (IgG or IgM) on a flow cytometer.

- Mixed-Passive Hemagglutination Assay (MPHA): Intact platelets are immobilized in round-bottom wells of a microtiter tray. A serum sample is added to the tray, followed by washing and addition of detector red cells previously coated with an antibody specific for human immunoglobulin. After incubation and centrifugation, the tray is examined visually for a positive reaction (evenly distributed red blood cells) or a negative reaction (red cell button in the center of the well).

Antigen-Capture Assays:

These assays use monoclonal antibodies that recognize target antigens of interest but do not compete with the platelet antibody being detected.

- The Monoclonal Antibody Immobilization of Platelet Antigens (MAIPA) Assay. This assay consists of target platelets, murine monoclonal antibodies and a microtiter tray containing immobilized goat antibody-specific for mouse IgG. Target platelets are sensitized with patient serum and murine IgG monoclonal antibodies. Platelets are washed and solubilized in a non-ionic detergent, and centrifuged to remove cytoskeletal fragments. An aliquot of the supernatant lysate is added to wells of the microtiter tray. The murine monoclonal antibody is captured and the platelet surface glycoprotein with its bound human antibody becomes immobilized. After a wash step, the human antibody is detected with an enzyme labeled goat anti-human immunoglobulin probe [4].

- Antigen-capture ELISA (ACE) techniques and modified antigen-capture ELISA (MACE). In ACE, platelet glycoproteins are bound to the plate by a specific monoclonal antibody. Patient serum is added and bound antibodies are detected

by a second enzyme-labeled anti-human antibody. In the MACE methods, patient serum is incubated with platelets first (prior to antigen capture), followed by platelet washing and detergent lysis. Surface glycoprotein with bound human antibody is then captured on the plate by monoclonal antibodies and antibodies are detected with an enzyme labeled goat anti-human immunoglobulin probe.

- Bead based assay: This assay uses beads coupled to platelet lysate glycoproteins carrying clinical relevant HPA specificities to capture and identify HPA antibodies. The beads are incubated with test serum, washed and a fluorochrome-conjugated anti-human immunoglobulin antibody is added. Antibody binding is measured by flow cytometry.

FDA has cleared HPA devices that use some of the technologies described above. The information about FDA-cleared devices is included in Appendix B, Literature Review.

1.3.3. HNA Devices

There are two categories of HNA devices: 1) HNA typing devices: for use in determining the HNA phenotype or genotype; 2) HNA antibody detection devices: for screening or identifying antibodies to HNA antigens. The devices may consist of all the necessary reagents in a kit, or be designed as a system consisting of reagents, instrument and software. Software used to support the evaluation and interpretation of HNA results may be submitted to the FDA as part of a system, or in a separate premarket submission. Since HNA devices are unclassified, there is no codified regulation associated with the HNA devices.

1.3.3.1. Human Neutrophil Antigen Typing Devices

Serologic testing for HNA is performed using characterized antisera or monoclonal antibodies specific to several HNA antigens. Genotyping has become a preferred method for HNA typing. The genotyping methods that are now available to predict human neutrophil antigens are similar to the methods used for HLA and HPA typing devices.

1.3.3.2. Human Neutrophil Antibodies Detection Devices

The classic serological methods for the detection of HNA antibodies include the following [6]:

- Granulocyte agglutination test (GAT): A panel of purified granulocytes from three to five donors are isolated and then incubated with the test serum or plasma sample. Granulocyte agglutination is evaluated using an inverted-phase microscope and graded from negative to 4+ based on the percentage of cells that are agglutinated. This method can detect both IgG and IgM antibodies.

- Granulocyte immunofluorescence test (GIFT): A panel of purified granulocytes from three to five donors is isolated and treated with paraformaldehyde. The cells are incubated with test serum or plasma, washed and incubated with fluorescent

conjugated anti-human immunoglobulin. Following another washing step, immunofluorescence reactions are detected with a microscope or by flow cytometry.

- Monoclonal antibody (MoAb)-specific immobilization of granulocyte antigens (MAIGA) assay: This method is mostly used for confirmation of GIFT and GAT results. The methodology is similar to the MAIPA assay described for the HPA antibody detection devices (See section 1.3.2.2).

- Bead-based assays: This assay uses beads coated with specific HNA antigens. The beads are incubated with test serum, washed and a fluorochrome-conjugated anti-human immunoglobulin antibody is added. Antibody binding is measured by flow cytometry.

1.3.4. HLA, HPA and HNA Device Components

The components of HLA, HPA and HNA devices may include:

- Reagents (e.g., antibodies, antigens, primers, probes, enzymes, buffers, etc.)
- Instrument(s) which may include operating software
- Data analyzing software, which may be submitted as a separate premarket notification 510(k).

1.4. HLA, HPA and HNA Devices Included for Consideration

We are seeking a recommendation for the classification of the following devices:

- HLA devices used in determining the HLA type (phenotype or genotype). The classification will be limited to devices used to aid donor-recipient matching in transplantation or transfusion, or to aid the diagnosis of a disease.
- HLA devices used in detecting antibodies to HLA antigens. The classification will be limited to devices used to aid donor-recipient matching in transplantation or transfusion.

- HPA devices used in determining the HPA type (phenotype or genotype) and for detecting antibodies to HPA antigens. The classification will be limited to devices used to aid in donor-recipient matching in blood transfusion, or to aid the diagnosis of a disease.

- HNA devices used in determining the HNA type (phenotype or genotype) and for detecting antibodies to HPA antigens. The classification will be limited to devices used to aid in donor-recipient matching in blood transfusion, or to aid the diagnosis of a disease.

The following devices are not included for consideration:

- HLA, HPA or HNA devices used as companion diagnostic device (device that provides information that is essential for the safe and effective use of a corresponding therapeutic product) [7].
- HLA, HPA or HNA assays that are intended for clinical use and designed, manufactured and used within a single laboratory.

2. Regulatory History

A summary of the regulatory history for HLA, HPA and HNA devices is provided below.

2.1. Previous Device Review and Clearance

2.1.1. HLA Devices

The first product license for Leukocyte Typing Serum was issued in December 1974, by the Bureau of Biologics, FDA. Leukocyte Typing Serum is prepared from blood or plasma of human donors or from animals and contains antibodies that are used to identify HLA antigens. An FDA guideline for the production, testing, and lot release of Leukocyte Typing Serum was issued in 1977 and subsequently codified as Additional Standards in the Biologics regulations under 21 CFR 660.10 through 660.15.

On August 1, 1980, the FDA published a proposed rule (45 FR 51226) recommending that the Additional Standards for Leukocyte Typing Serum be removed with the subsequent revocation of the existing product licenses. The proposal was prompted by the realization of the growing complexities of the HLA system and the difficulty in achieving standardization. The proposal was supported by the argument that the products, while biologics, were also medical devices which could be appropriately regulated under the Federal Food, Drug, and Cosmetic Act as amended by the Medical Device Amendments of 1976 (21 U.S.C 301 et seq). The agency's intent to classify HLA reagents and kits was described in the preamble to the 1980 proposed rule.

On August 10, 1982, FDA published a final rule [8] removing the additional standard for Leukocyte Typing Serum. It instructed all manufacturers of Leukocyte Typing Serum to register and list under 21 CFR 807, and if not currently licensed, submit premarket notifications (510(k) submissions).

At the September 15, 2000 Blood Products Advisory Committee (BPAC) meeting, the committee serving as a device classification panel provided recommendations to FDA regarding the classification of in vitro diagnostic reagents and kits for use in determining the HLA phenotype or genotype of an individual, or for detecting antibodies to HLA antigens. The scope of the discussion included devices which are used to support platelet and leukocyte transfusions, or organ and stem cell transplantation. The classification of HLA kits used to predict disease was not discussed at the meeting. The BPAC unanimously agreed (13 yes – 0 no – 0 abstain) that HLA devices should be classified as Class II medical devices. The panel did not agree (0 yes – 13 no – 0 abstain) that the devices should be exempt from

the requirement to submit a 510(k) [9]. Although the BPAC recommended classification of the HLA devices as Class II, CBER has not proceeded with rulemaking.

Between 1982 and 2017, FDA has cleared approximately 100 HLA device premarket notifications (510(k)) submissions. The 510(k) review is to ensure substantial equivalence to the predicate device. Among others, FDA has reviewed the following main elements in an HLA device 510(k) submission:

- Comparison of intended use and technology to the predicate
- Accuracy study (as appropriate)
- Interference testing
- Limit of detection
- Clinical comparison study (at least three sites)
- Precision Studies including repeatability and reproducibility
- Stability studies
- Software validation (if applicable)
- Validation of Instrumentation (if applicable)
- Labeling

FDA believes the premarket review process provides an oversight of the HLA devices that will help ensure the generation of correct testing results.

2.1.2. HPA Devices

Since 1993, FDA has cleared seven HPA assays through the 510(k) premarket notifications pathway. Five devices were cleared for the detection of antibodies against platelet glycoprotein antigens and two were cleared for platelet antigen determination.

2.1.3. HNA Devices

Since 2006, FDA has cleared four HNA devices through the 510(k) premarket notifications pathway. Two devices were cleared for the detection of antibodies against neutrophil antigens and two were cleared for determination of neutrophil antigens.

2.2. FDA Guidance on HLA devices

In July 2015, FDA issued guidance regarding 510(k) submissions for HLA genotyping kits used in transfusion and transplantation- “FDA Guidance for Industry: Recommendations for Premarket Notification (510(k)) Submissions for Nucleic Acid-Based Human Leukocyte Antigen (HLA) Test Kits Used for Matching of Donors and Recipients in Transfusion and Transplantation” [10].This guidance document provides recommendations to submitters and FDA reviewers in preparing and reviewing 510(k) submissions for molecular based HLA devices, and gives detailed information on the types of studies FDA recommends for validation of HLA genotyping kits. FDA states in this guidance that some of the recommendations in the guidance may be applicable to HNA and HPA test kits.

2.3. Cleared Intended Use

A device's Intended Use statement provides a general description of the diseases or conditions that the device will diagnose, treat, prevent, cure, or mitigate, including a description, where appropriate, of the patient population for which the device is intended. Representative Intended Use for HLA, HPA and HNA devices cleared through the 510(k) premarket notifications pathway include:

HLA typing devices (genotype)

- DNA typing of Class I and Class II HLA Alleles
- Provides the HLA-A, HLA-B, HLA-C, HLA-DRB and HLA-DQB typing results with a low to medium resolution by a DNA-based method
- A PCR based HLA typing evaluation method designed to provide resolution of the HLA Class I (A, B, C) and Class II (DRB, DQB) loci using genomic DNA
- DNA Typing of Class I or Class II HLA alleles, to aid in transfusion and transplantation donor and recipient matching.

HLA typing devices (phenotype)

- For use in determining HLA Class I and Class II cell surface antigens with a complement dependent microlymphocytotoxic technique
- For the detection of HLA-B27 antigen expression. The presence of HLA-B27 antigen is strongly associated with ankylosing spondylitis (AS) and a few other rheumatic disorders.

HLA antibody detection devices

- For use in detection of HLA antibodies using flow cytometric technology
- Qualitative bead-based immunoassay used to detect IgG antibodies to HLA Class I and Class II molecules
- For the detection of antibodies to human leukocyte antigens
- To detect anti-HLA class I and class II antibodies in human serum or plasma of blood donors

Software devices used to assign/evaluate results

- An accessory for the evaluation of test results from [company/product name] HLA antibody detection products
- To support evaluation of HLA typing results

HPA typing devices

- Genotyping of HPA-1 (Pl^A)
- Molecular determination of HPA-1 (PLA^A), HPA-2, (Ko), HPA-3 (Bak), HPA-4 (Pen), HPA-5 (Br), HPA-6 (Ca), and HPA-15 (Gov), using PCR amplification of human genomic DNA.

HPA antibody detection devices

- Detection of IgG antibodies to platelet specific antigens.

- Detection of antibodies to platelet glycoproteins (GPs) IIb/IIIa, Ib/IX, Ia/IIa, and IV.
- Screening test for autoantibodies to platelet GPs IIb/IIIa, Ib/IX, and Ia/IIa.
- Screening of antibodies to platelet GP IV antigens, and to polymorphic epitopes on the platelet GPs IIb/IIIa, Ib/IX and Ia/IIa.

HNA typing devices

- Molecular determination of neutrophil polymorphisms – HNA 1a, 1b, 1c, 3a, 3b, 4a, 5a, and 5b.
- Molecular determination of alleles of neutrophil polymorphisms HNA-1: HNA-1a (NA1), HNA-1b (NA2), and HNA-1c (SH).

HNA antibody detection devices

- Luminex based antibody detection assay to detect anti-HLA class I/class II antibodies and anti-HNA antibodies.

3. Clinical Background

3.1. HLA Devices

3.1.1. HLA testing and donor-recipient matching in transplantation

HLA testing is used to aid donor and recipient matching in transplantation. A major factor contributing to the success of many transplants is the prevention of rejection due to immune responses of the recipient to the donor tissue (alloimmunization). One of the key strategies to reduce rejection is to minimize HLA differences between the donor and recipient. HLA testing is widely utilized in transplantation. For example, the United Network for Organ Sharing (UNOS) requires that kidney donors and recipients are prospectively typed for HLA antigens [11]. Data continue to support HLA matching in kidney transplantation [12]. The selection of a related or an unrelated hematopoietic stem cell donor for a patient requires HLA matching to maximize the beneficial effects of the transplant. Furthermore, pre-transplant donor-specific HLA antibodies in the recipient are often associated with an increased risk of rejection and graft loss in solid organ transplants [13]. Therefore, identification of donor-specific HLA antibodies allows for informed decisions regarding whether to accept and transplant an organ for a specific recipient.

3.1.2. HLA testing and transfusion

In the transfusion setting, HLA devices are used to screen donors, obtain compatible units, or investigate transfusion reactions. Some of the adverse reactions observed in recipients of blood components are triggered by HLA antigens or antibodies.

- Transfusion related acute lung injury (TRALI) is typically associated with the presence of HLA antibodies in plasma-containing blood components. This condition continues to be a leading cause of transfusion-related fatalities reported to FDA [14]. Measures to mitigate the occurrence of TRALI include testing donors for HLA antibodies [15]. HLA antigen

and antibody testing continues to be essential for the investigation of TRALI in blood transfusion recipients.

- HLA antibodies in patients can cause immune-mediated platelet refractoriness (IPR), and failure to respond to platelet transfusion therapy. HLA testing is commonly performed on both the donor and recipient to obtain compatible platelets for patients with IPR[16].
- In susceptible recipients, HLA reactive cells in a cellular product can induce transfusion-associated graft versus host disease (TA-GVHD). Investigation of TA-GVHD includes testing of the donor and recipient HLA antigens [17].

3.1.3. HLA testing and disease diagnosis

In parallel with our increased understanding of the genetic complexity of the HLA system and its extensive polymorphisms, many diseases have now been reported to occur more frequently in individuals with particular HLA types. These diseases include a broad spectrum of immune-mediated diseases and malignancies [18]. HLA testing has also been used to assist disease diagnosis. For example, HLA-DQA1 and HLA-DQB1 loci have been identified as the main genetic factors for celiac disease [19]. HLA-DQ genotyping can be used to screen asymptomatic children and in cases of histology/serology disagreement. HLA-B27 typing is recommended to assist in the diagnosis of several immune disorders or inflammatory diseases [20].

3.2. HPA testing and transfusion

In the transfusion setting, HPA devices are used to diagnose disease, screen donors, obtain compatible or matched platelet units, or investigate transfusion reactions.

- Fetal and neonatal allo-immune thrombocytopenia (FNAIT) is the most common cause of moderate and severe thrombocytopenia in the fetus or an otherwise healthy newborn [28]. FNAIT may lead to severe bleeding complications, such as intracranial hemorrhage. Thrombocytopenia in FNAIT is caused by maternal alloantibodies against human platelet antigens resulting from maternal alloimmunization after exposure to paternally derived antigens on fetal platelets. The most commonly involved are HPA-1a alloantibodies, which are responsible for ~ 80% of FNAIT cases [29,30]. HPA testing is used to support the diagnosis of FNAIT and test potential platelet donors for compatible platelet units for transfusion [3].

- The presence of circulating HPA antibodies in patients can cause immune-mediated platelet refractoriness (IPR) and failure to respond adequately to platelet transfusion therapy [31]. HPA testing is commonly performed on both the donor and recipient to obtain compatible platelets for transfusion.

- Post-transfusion purpura (PTP) is an uncommon, but potentially life threatening, delayed transfusion complication characterized by profound thrombocytopenia and purpura. PTP is caused by anti-HPA antibodies formed following sensitization from transfusion or pregnancy [32,33]. Anti-HPA-1a alloantibodies are most commonly associated with the

disease, although antibodies against HPA-1b, HPA-2b, HPA-3a, HPA-3b, HPA-4a, and HPA-5b have also been implicated [34]. HPA testing is used to support the diagnosis of PTP.

3.3. HNA testing and transfusion

Transfusion related acute lung injury (TRALI) occurs when a susceptible patient receives a blood component that triggers the reaction through exposure to either alloantibodies against HLA antigens, or HNA antigens or biologic response mediators [35-37]. While TRALI is typically associated with the presence of HLA antibodies, antibodies to neutrophil antigens have been implicated. Antibodies to HNA-1a, HNA-1b, HNA-2a, and HNA-3a have been associated with TRALI. HNA-3a antibodies are the most frequently detected of the HNA antibodies in TRALI cases, seen in up to 85% of the cases [38]. HNA antigen and antibody testing continues to be essential for the investigation of TRALI in blood recipients [39].

3.3.1. Other clinical uses of HNA Testing

- Neonatal alloimmune neutropenia (NAN) is an uncommon but life threatening disease of the newborn. It is caused by maternal sensitization to foreign neutrophil antigens and development of neutrophil IgG antibodies [40]. The antibodies cross the placenta and destroy fetal neutrophils. Newborns with NAN are susceptible to infections. HNA devices are used to support the diagnosis of NAN.

- Autoimmune neutropenia (AIN) may be idiopathic or secondary to an underlying disease. Primary AIN is the most common cause of chronic neutropenia in infants. HNA devices may be used to investigate autoimmune thrombocytopenia.

4. Systematic Literature Review on HLA devices, HPA devices and HNA devices

FDA conducted a literature review of the safety and effectiveness of HLA, HPA and HNA devices, to identify valid scientific evidence related to benefits and risks of these medical devices.

4.1. HLA Devices

The scope of the HLA device systematic literature review addressed the following questions:

- What is the reported analytical performance of the FDA-cleared HLA devices (HLA typing devices and HLA antibody detection devices)?

- How are the HLA testing results used to aid donor-recipient matching in transfusion or transplantation, or to aid disease diagnosis?

- What are the risks associated with HLA devices?

The literature search was conducted through PubMed and EMBASE for articles published from January 1, 1994 to May 1, 2017 for the analytical performance of HLA devices and the use of HLA testing results for donor and recipient matching. We selected HLA-B27 and its association with ankylosing spondylitis (AS) as an example of the safety and effectiveness of HLA assays used to aid in disease diagnosis. The related literature search was performed for articles published from January 1, 2011 to May 1, 2017. The specific methods used and exclusion criteria are discussed in detail in the full report (Appendix A).

Our systematic review of relevant literature pertaining to HLA devices is summarized below:

1. Analytical performance of the HLA typing and HLA antibody detection devices

   Our literature review included studies that evaluated the analytical performance of FDA-cleared HLA devices used in the setting of transplantation and transfusion. These studies provide important information regarding assay specificity, reproducibility, comparability and potential interference for HLA antibody detection devices.
   Overall, the studies showed that these devices performed comparably with some showing advantage over others in terms of sensitivity and specificity. Some factors, such as denatured HLA antigens presented on the beads, prozone effect and interferences, may affect HLA antibody detection. Because of the widespread polymorphism of the HLA system, test results may be variable. In addition, HLA typing ambiguities remains as a major challenge with many devices. Apparent false negative or false positive HLA molecular typing results may need further investigation to rule out possible new alleles.

2. Clinical use of HLA devices in transfusion, transplantation and disease diagnosis

   HLA assays have been widely used to aid donor and recipient matching in transfusion and transplantation. The studies generally revealed that HLA assays are important in matching of donors and patients, mitigating transfusion reactions such as TRALI, and for the diagnosis of diseases such as Ankylosing Spondylitis. We identified relatively few studies evaluating the use of FDA-cleared HLA typing devices in clinical settings. While it is recognized that other factors influence clinical outcome, these studies showed that HLA mismatch negatively impacted patient outcome. Many other studies examined the association of preformed HLA antibodies and clinical outcome. In general, the presence of HLA antibodies in patients or donors, especially donor-specific antibodies (DSA), is associated with worse clinical outcomes. In addition, identifying and considering HLA antibodies with no clinical impact may lead to unjustified exclusion of potential donors.

   Due to the role of the immune system in many diseases, certain HLA types are associated with an increased risk for disease. The association of HLA-B27 and AS provides a good example of the use of HLA test results to support disease diagnosis. Incorrect HLA-B27 test results may affect or delay AS diagnosis.

3. Risks associated with HLA devices

While studies showed acceptable analytical performance of HLA devices and widespread clinical use, several device limitations identified may cause false positive or false negative HLA test results. Such results could lead to adverse patient outcomes, poor graft survival, transfusion reactions, or incorrect or delayed diagnosis of related conditions.

Based on our systematic review of the literature, we found studies that examined the analytic performance and clinical use of HLA devices and their associated risks. HLA devices play an important role to aid donor and recipient matching and disease diagnosis. Device limitations can lead to incorrect HLA testing results that may negatively affect patient care. As such, it would appear that continued review of premarket notification [510(k)] submissions is necessary to demonstrate a reasonable assurance of safety and effectiveness.

3.1. HPA and HNA Devices

The scope of the review addressed the following questions:

- What is the reported analytical performance of the HPA and HNA devices?
- What are the reported clinical uses of the HPA and HNA testing results?
- What are the risks associated with HPA and HNA devices

The literature search was conducted through PubMed and EMBASE for articles published from January 1, 1994 to May 1, 2017. The specific methods used are discussed in detail in the full report (Appendix B).

1. Analytical performance of the HPA and HNA typing and antibody detection devices

Our literature review did not identify any studies that evaluated the analytical performance of FDA-cleared HPA typing or HPA antibody detection devices. Similarly, there were no studies that evaluated the analytical performance of the FDA-cleared HNA typing devices. However, our literature review included a small number of studies that evaluated the analytical performance of the only FDA-cleared HNA antibody detection device.

Overall, the studies showed that the FDA-cleared HNA antibody detection device performed comparably with flow cytometry, granulocyte agglutination (GAT) paired with monoclonal antibody-specific immobilization of granulocyte antigens (MAIGA) and other classical non-FDA-cleared tests. Differences in performance were noted depending on whether the disease was mediated by an auto- or alloimmune process, with concordance appearing to be better for alloimmune conditions compared to autoimmune conditions. It was also noted that the FDA-cleared HNA antibody detection device was less reliable than GAT, GIFT and MAIGA for differentiating HNA antibody specificity.

2. Clinical use of HPA and HNA devices in transfusion and disease diagnosis

Testing results from HPA devices have been widely used to diagnose disease in the setting of fetal or neonatal thrombocytopenia, and to select appropriate antigen-negative platelet products for transfusion. HPA devices are also used to evaluate the etiology of platelet refractoriness in

patients who have been frequently transfused and for selection of appropriate products for transfusion when platelet antibodies are present.

In the transfusion setting, HNA devices are used to evaluate adverse reactions in transfusion recipients and to mitigate the risk of TRALI potentially caused by blood donors with HNA antibodies. Other less frequent clinical uses described in the literature include use in diagnosing neonatal alloimmune neutropenia, autoimmune neutropenia and drug-induced neutropenia.

3. Risks associated with HPA and HNA devices

The literature review showed that use of the FDA-cleared HPA and HNA devices play an important role in transfusion and transplantation and disease diagnosis. However, device malfunction can occur causing false positive or false negative results. Spurious results may affect patient outcomes through delayed or missed diagnoses, delayed treatment and selection of inappropriate or ineffective products for transfusion.

Conclusions

Based on our systematic review of the literature, we found that FDA-cleared HPA and HNA devices demonstrate acceptable analytical performance (in the case of HNA antibody detection), and play a critical role aiding in disease diagnosis. Device limitations can lead to incorrect HPA and HNA testing results that may negatively affect patient care.
As such, continued review of premarket notification [510(k)] submissions are necessary to demonstrate a reasonable assurance of safety and effectiveness.

5. Medical Device Reports (MDRs) and Product Recall Reports

5.1. Overview of MAUDE Database

The Manufacturer and User Facility Device Experience (MAUDE) database is maintained by the Office of Surveillance and Biometrics at FDA. This database houses medical device reports (MDRs) submitted to the FDA by mandatory reporters (manufacturers, importers and device user facilities) and voluntary reporters (health care professionals, patients and consumers). Mandatory and voluntary reporters submit MDRs of suspected device-associated deaths, serious injuries and malfunctions. The FDA uses MDRs to monitor device performance, detect potential device-related safety issues, and contribute to benefit-risk assessments of these products.

Although MDRs are a valuable source of information, this passive surveillance system has limitations, including the potential submission of incomplete, inaccurate, untimely, unverified, or biased data. Not all events are captured since reporting is mandatory for manufacturers and user facilities, but voluntary for consumers and healthcare practitioners. In addition, confirming whether a device caused a specific event can be difficult based solely on information provided in each report.

5.2. MAUDE Search Results:

5.2.1. HLA devices

FDA conducted queries of the MAUDE database to identify MDR reports related to the use of HLA devices. The search was restricted to all reports that FDA received and entered into the database before May 1, 2017, and utilized the product code MZI. There were 477 MDRs for HLA devices. Most MDRs (464) were reported for HLA genotyping devices (Table 2). Thirteen (13) MDRs were reported for HLA antibody detection devices (Table 3). Note that a specific issue may affect multiple products/kits and/or multiple lots, resulting in multiple MDRs. For example, one probe/primer may be used in multiple kits so a problem with this component will affect multiple kits.

Table 2: MDR received by reportable event types by year for HLA genotyping devices

Report Year	Death	Injury	Malfunction	Not identified**	Totals
2012	0	0	5	0	5
2013	0	0	133	29	162
2014	0	0	199	1	200
2015	0	0	97	0	97
2016	0	0	0	0	0
2017*	0	0	0	0	0
Totals	0	0	434	30	464

*Until May 1, 2017

** Submitter did not identify a reportable event type.

Table 3: MDR received by reportable event types by year for HLA antibody detection devices

Report Year	Death	Injury	Malfunction	Not identified**	Totals
2012	0	0	0	0	0
2013	0	0	0	0	0
2014	0	0	7	0	7
2015	0	0	0	0	0
2016	0	0	2	0	2
2017*	0	0	0	4	4
Totals	0	0	9	4	13

*Until May 1, 2017

** Submitter did not identify a reportable event type

Review of the MDRs identified the following major issues regarding HLA genotyping devices:

- Incorrect reactivity assignment for primers/probes. Identified root causes include:
  - errors when updating software or datasheet with new HLA allelic information

    - errors related to internal manufacturing software that contains reactivity assignment information
    - lack of sample(s) to verify all possible reactivity assignments before product release
- Malfunctions of data interpretive software.
- Malfunctions of Sanger Sequencing kits due to high sequencing background or poor sequencing quality.

Table 4 provides more details on the identified issues sorted by the number of associated reports:

Table 4: Identified issues and root cause associated with HLA genotyping device MDR reports

Identified Issues	Root Cause	# of MDRs
Incorrect reactivity assignment for primers/probes	Root cause not identified.	239
	Errors when updating software/data sheet with new HLA allelic information	67
	Errors related to internal manufacturing software that contains reactivity assignment information	57
	Samples of rare/new alleles had not been tested to verify reactivity assignments before product release	8
Issues related with data interpretive software	"size filter" (a function limiting results to certain alleles based on reaction pattern) malfunction	36
	Incorrect serologic equivalent information in the software database	3
	software bug	1
	incorrect pseudogene labeling	1
Mistype or no type	Root cause not identified	16
PCR primer false reactions resulted mistype or no type	Root cause not identified	2
High sequencing background or poor sequencing quality	Root cause information is not available	24
Failed or weak amplification	Ice crystals in trays	2
	Degraded buffer	1
	Root cause not identified	1
Incorrect serologic equivalent designation	internal software bug	2
	Root cause not identified	1
Error in allele update worksheet, allele amplification imbalance or user error	No further information was provided	3

Overall, the most frequent malfunctions reported for HLA genotyping devices are incorrect reactivity assignments that lead to mistype or no type results. Failing to deliver correct HLA typing results poses potential risks to patients (see Section 6). Mitigation of such risks caused by incorrect assignments usually requires accurate reactivity labeling for primers/probes, and correct updating of the allele database, the data interpretation worksheet and the software when applicable.

Issues and root cause reported in the MDRs for HLA antibody detection devices
All of the MDRs related to HLA antibody detection devices were categorized as "Malfunction" or left blank in the Event Report Type field (Table 3). The following issues and root causes were identified:

- False positive results or unusually high positive rates – five (5) MDRs. Identified root causes include:
    - Low assay cutoff
    - Inconsistent results between lots, especially for samples with reactivity near the assay cutoff
- False negative results – four (4) MDRs. The root cause was failure to detect antibodies of low affinity and/or low titer which are specific for low frequency HLA epitopes.
- Inconsistent results from two runs – two (2) MDRs. The discrepancy may be attributed to increased background in the negative control reading.
- Negative serum control did not meet QC criteria – two (2) MDRs.

Failing to provide correct HLA antibody results poses potential risks to patients (see Section 6). Mitigation of such risks usually requires performance evaluation including testing samples with HLA antibody levels around assay cutoff and precision studies.

5.2.2. HPA and HNA devices

Compared to HLA devices, there are very few FDA-cleared HPA and HNA devices in the U.S. market. FDA conducted queries of the MAUDE database to identify MDR reports related to the use of HPA and HNA devices. The search was restricted to the period January 1, 1991 to May 1, 2017, and utilized the product code MYP. There were two MDRs for HPA devices and no MDRs for HNA devices.

The two MDRs related to HPA devices were categorized as "Malfunction." These were related to false positive results. Root cause investigations revealed the following issues:

- Several steps in the assay were not being performed according to the product instructions for use (IFU). No failure was detected in the investigation and it was confirmed that the device operated within specifications.
- A false positive result could not be replicated in-house and no failure was detected. It was confirmed that the device operated within specifications.

5.2.3. Conclusion

HLA device malfunctions reported through MAUDE have the potential to cause adverse health consequences. These medical device reports suggest that the current requirement for premarket notification [510(k)] review of HLA devices is an effective means of minimizing adverse health consequences that may result from HLA device malfunctions.

There are few HPA and HNA devices in the U.S. market and few reported MDRs. However, these devices share similar technologies and clinical applications to HLA devices and have the potential for malfunctions that may cause adverse health consequences. Therefore, the current requirement for premarket notification [510(k)] review of HPA and HNA devices is needed to minimize adverse health consequences that may result from HPA or HNA device malfunction.

5.3. HLA, HPA and HNA device recalls

5.3.1. Overview of Product Recalls

A recall is an action taken to address a problem with a marketed medical device that violates FDA law. Recalls occur when a medical device is defective, when it could be a risk to health, or when it is both defective and a risk to health. A recall is either a correction or a removal depending on where the action takes place. Note that classification of a recall as Class I, II, or III is unrelated to the classification of medical devices. Information on device recalls can be obtained from FDA's Medical Device Recalls database.

Recalls are classified as follows:

- Class I recall: a situation in which there is a reasonable probability that the use of, or exposure to a violative product will cause serious adverse health consequences or death.

- Class II recall: a situation in which use of, or exposure to, a violative product may cause temporary or medically reversible adverse health consequences or where the probability of serious adverse health consequences is remote.

- Class III recall: a situation in which use of, or exposure to, a violative product is not likely to cause adverse health consequences.

5.3.2. HLA, HPA and HNA device recall search results

FDA searched the Medical Device Recalls database for all recalls received until May 1, 2017 and related to HLA devices. Since 2009, medical device manufacturers have initiated a total of 37 recalls for HLA devices. There were no recalls for HPA and HNA devices. Of the 37 HLA device recalls, none were classified as Class I recalls, 19 were classified as Class II, and 18 were classified as Class III. Most of the recalls (32 out of the total 37, including three recalls for antibody detection kits) were for products that failed to provide correct testing results (false negative, false positive, mistype or no type) (Table 5). The root causes leading to incorrect HLA typing results include incorrect reactivity assignments, lack of testing sample(s) with specific allele before releasing, manufacturing errors, etc. The HLA antibody

device recalls were due to manufacturing errors during the production of recombinant HLA proteins, such as unstable transfectant.

Table 5. HLA device recalls

Recall class	Failed to provide correct results			Other issues			Total
	HLA typing devices	HLA Ab detection devices	software	HLA typing devices	HLA Ab detection devices	software	
Class I	0	0	0	0	0	0	0
Class II	17	0	0	0	2	0	19
Class III	11	3	1	3	0	0	18

5.3.3. Conclusion

There have been 37 recalls for HLA devices and none for HPA and HNA devices. All the recalls related to HLA devices were classified either as Class II or Class III by FDA. There were no Class I recalls in which the violative product could cause serious adverse health consequences or death. No recalls were reported for HPA and HNA devices. However, these devices share similarities with the HLA devices and likely are prone to similar malfunctions. The recall analysis suggests that the current requirement for premarket notification [510(k)] review of HLA devices is a necessary means to minimize adverse health consequences that may result from the use of these devices.

6. Risks to Health

The risks to health associated with HLA, HPA and HNA devices include but are not limited to the following:

HLA, HPA and HNA devices can malfunction, causing mistypes, and false positive or false negative results. The failure to deliver correct HLA, HPA and HNA testing results can result in adverse health consequences including but not limited to:

Patient injury or death due to:

a. Poor graft survival or function due to transplantation of incompatible hematopoietic cells, tissue or organ.
b. Graft rejection because of the transplantation of incompatible hematopoietic cells, tissue or organ.
c. Graft-versus-host disease because of the transplantation of incompatible lymphocytes.
d. Incorrect or delayed diagnosis of related conditions.
e. Transfusion reaction (e.g. TRALI, PTP) due to incorrect HLA, HPA or HNA test results.

f. Platelet refractoriness because of incorrect HLA or HPA typing or antibody detection results.

The panel will be asked to comment on the risks to health that have been identified by FDA, whether these risks are appropriate, and whether there are additional risks to health that should be considered for HLA devices, HPA devices and HNA devices.

7. Elements for Consideration for the Device Panel

Based on the available information, the Panel will be asked to comment on whether HLA, HPA, and HNA devices meet the statutory definition associated with a Class III device:

> The Federal Food, Drug, and Cosmetic Act, section 513, established the risk-based device classification system for medical devices. A device is classified as class III because it:
>
> (i)
>
> (I) cannot be classified as a class I device because insufficient information exists to determine that the application of general controls are sufficient to provide reasonable assurance of the safety and effectiveness of the device, and
>
> (II) cannot be classified as a class II device because insufficient information exists to determine that the special controls would provide reasonable assurance of its safety and effectiveness, **and**
>
> (ii)
>
> (I) is purported or represented to be for a use in supporting or sustaining human life or for a use which is of substantial importance in preventing impairment of human health, or
>
> (II) presents a potential unreasonable risk of illness or injury,
>
> Class III devices are subject to premarket approval to provide reasonable assurance of its safety and effectiveness.

If HLA, HPA, and HNA devices do not meet the statutory definition of a Class III device, the panel will be asked whether these mentioned devices would more appropriately be classified as Class II devices. FDA classifies a device as class II for which General Controls alone are insufficient to provide reasonable assurance of the safety and effectiveness of the device, and for which there is sufficient information to establish Special Controls to provide such assurance. Special Controls are usually device-specific.

If the panel decides that HLA, HPA, and HNA devices are neither class III nor class II devices, the panel, therefore, believes that General Controls alone are adequate to ensure a reasonable

assurance of safety and effectiveness for HLA, HPA, and HNA devices and should be classified as a Class I medical device.

For the purposes of classification, FDA considers the following items, among other relevant factors, as outlined in 21 CFR 860.7(b):

1. The persons for whose use the device is represented or intended;

2. The conditions of use for the device, including conditions of use prescribed, recommended, or suggested in the labeling or advertising of the device, and other intended conditions of use;

3. The probable benefit to health from the use of the device weighed against any probable injury or illness from such use; and

4. The reliability of the device.

7.1. Indications for Use

FDA regulations require that a device description include the indications for use. Indications for use is a general description of the disease or condition the device will diagnose, treat, prevent, cure, or mitigate, including a description of the patient population for which the device is intended.

FDA is recommending the following descriptions in proposed regulations for HLA, HPA and HNA devices:

- Indications for Use for HLA typing devices: To be used to determine {indicate HLA locus, loci or antigens}, to aid donor and recipient matching in transfusion or transplantation, or to aid the diagnosis of {indicate disease}.
- Indications for Use for HLA antibody detection devices: To be used to detect antibodies to {list of HLA antigens}, to aid donor and recipient matching in transfusion or transplantation.
- Indications for Use for HPA typing devices: To be used for the detection of human platelet antigens {indicate HPA antigens}, to aid donor and recipient matching in transfusion, or to aid the diagnosis of {indicate disease}
- Indications for Use for HPA antibody detection devices: To be used to detect antibodies to {list of HPA antigens}, to aid donor and recipient matching in transfusion, or to aid the diagnosis of {indicate disease}.
- Indications for Use for HNA typing devices: To be used for the detection of human neutrophil antigens {indicate HPA antigens}, to aid donor and recipient matching in transfusion, or to aid the diagnosis of {indicate disease}.

- Indications for Use for HNA antibody detection devices: To be used to detect antibodies to {list of HNA antigens}, to aid donor and recipient matching in transfusion, or to aid the diagnosis of {indicate disease}.

7.2. Valid Scientific Evidence

In making a recommendation regarding the safety and effectiveness of HLA, HPA and HNA devices, the panel will be asked to consider "valid scientific evidence." According to 21 CFR 860.7(c)(2), "Valid scientific evidence is evidence from well-controlled investigations, partially controlled studies, studies and objective trials without matched controls, well-documented case histories conducted by qualified experts, and reports of significant human experience with a marketed device, from which it can fairly and responsibly be concluded by qualified experts that there is reasonable assurance of the safety and effectiveness of a device under its conditions of use. The evidence required may vary according to the characteristics of the device, its conditions of use, the existence and adequacy of warnings and other restrictions, and the extent of experience with its use. Isolated case reports, random experience, reports lacking sufficient details to permit scientific evaluation, and unsubstantiated opinions are not regarded as valid scientific evidence to show safety or effectiveness. Such information may be considered, however, in identifying a device the safety and effectiveness of which is questionable."

7.3. Reasonable Assurance of Safety

According to 21 CFR 860.7(d)(1), "There is reasonable assurance that a device is safe when it can be determined, based upon valid scientific evidence, that the probable benefits to health from use of the device for its intended uses and conditions of use, when accompanied by adequate directions and warnings against unsafe use, outweigh any probable risks. The valid scientific evidence used to determine the safety of a device shall adequately demonstrate the absence of unreasonable risk of illness or injury associated with the use of the device for its intended uses and conditions of use."

A reasonable assurance of safety exists if, when using the device properly:

a. The probable benefits to health outweigh the probable risks; and

b. There is an absence of unreasonable risk of illness or injury.

The panel will be asked to comment on whether the available valid scientific evidence as discussed in Sections 3, 4, 5 and 6 demonstrates a reasonable assurance of safety for the indications for use described above.

7.4. Reasonable Assurance of Effectiveness

According to 21 CFR 860.7(e)(1), "There is reasonable assurance that a device is effective when it can be determined, based upon valid scientific evidence, that in a significant portion of the target population, the use of the device for its intended uses and conditions of use,

when accompanied by adequate directions for use and warnings against unsafe use, will provide clinically significant results."

The panel will be asked to comment on whether the available valid scientific evidence as discussed in Sections 3, 4, 5 and 6 demonstrates a reasonable assurance of safety and effectiveness for the indications for use described above.

8. FDA Summary

8.1. Summary of Valid Scientific Evidence

Available scientific evidence discussed in Sections 3, 4, 5 and 6 indicates that the safety and efficacy of HLA, HPA and HNA devices are important in the setting of transplantation and transfusion, and for disease diagnosis. HLA matching between the donor and recipient is a key strategy to reduce rejection. The presence of anti-HLA antibodies, especially donor-specific antibodies, has been associated with worse outcomes after transplantation or transfusion. Identification of HLA antibodies allows for informed decisions regarding whether to accept and transplant an organ for a specific recipient. In similar fashion, HPA and HLA devices provide a means to detect and identify related antigens and antibodies facilitating transfusion with compatible blood (platelet) products. In addition, HNA and HLA devices provide laboratorians and clinicians tools to investigate TRALI reactions and/or mitigate the risk of future TRALI reactions associated with implicated blood donors.

The literature review indicates that the use of these devices has improved patient care in transfusion and transplantation, and in disease diagnosis. The benefits to health from use of the devices outweigh any probable risks. Considering the current FDA 510(k) review requirements, FDA guidance document requests, proficiency testing program and quality control procedures, FDA believes that there is a reasonable assurance of efficacy for the use of HLA, HPA and HNA devices in transfusion, transplantation and as an aid in disease diagnosis.

Available literature, medical device reports, and medical device recall data also indicate that these complex devices can malfunction. These devices may generate false positive, false negative or inconsistent results. Therefore, device design, labeling, and device performance requirements are needed to ensure the safety and effectiveness of the HLA, HPA and HNA devices.

8.2. Special Controls

Due to the risk associated with these complex devices, FDA believes that General Controls alone are not sufficient to ensure the safety and effectiveness of HLA, HPA and HNA devices. FDA also believes that HLA, HPA and HNA devices are not life-supporting or life-sustaining, but are of substantial importance in preventing impairment of human health. The

available valid scientific evidence supports a Class II determination. FDA proposes the following Special Controls in addition to the General Controls to mitigate the risks to health and to provide a reasonable assurance of the safety and effectiveness of HLA, HPA and HNA devices.

Table 6: Risk/mitigation recommendations for HLA, HPA and HNA devices

Identified Potential Risk	**Recommended Mitigation Measure**
Inaccurate test results (i.e., false positive or false negative results) can result in adverse health consequences.	Special controls (1) (2) (3) (4) (5) (6) (7) (8) (9)
Failure of software to correctly interpret test results can result in adverse health consequences.	Special controls (5) (6)

When developing the Special Controls for HLA, HPA and HNA devices, we have considered the recommendations provided in FDA guidance "Recommendations for Premarket Notification (510(k)) Submissions for Nucleic Acid-Based Human Leukocyte Antigen (HLA) Test Kits Used for Matching of Donors and Recipients in Transfusion and Transplantation"[10].

Premarket notification submissions must include detailed documentation of the following information:

1. Device accuracy study using well-characterized samples representing as many targets as possible.
2. Precision studies to evaluate possible sources of variation that may affect test results.
3. Comparison studies to evaluate the device's performance compared to a predicate.
4. Specific information that address or mitigate risks associated with false positive antibody reactivity e.g., reactivity with denatured/cryptic epitopes, if applicable.
5. Description of how the assay cut-off was established and validated as well as supporting data.
6. Documentation for device software, including, but not limited to, software requirement specifications, software design specification, e.g., algorithms, alarms and device limitations; hazard analysis, traceability matrix, verification and validation testing, unresolved anomalies, hardware and software specifications; electromagnetic compatibility and wireless testing.
7. For multiplex assays in which large numbers of probes and/or primers are handled during the manufacturing process, premarket submissions should provide the design specifications that are in place to prevent incorrect reactivity assignment.
8. Description of a plan on how to ensure the performance characteristics of the device remain unchanged over time when new HLA alleles are identified, and/or reactivity assignments are changed from the assignments at the time the device was evaluated.
9. The device labeling must include:
    a. A limitation statement that reads, "The results should not be used as the sole basis for making a clinical decision."

b. A warning that reads “The use of this device as a companion diagnostic has not been established.”

If the panel believes that Class II is appropriate for HLA devices, HPA devices and HNA devices, the panel will be asked whether the identified special controls appropriately mitigate the identified risks to health and whether additional or different special controls are recommended.

8.3. FDA Recommendation

Based on the safety and effectiveness information outlined above, and the identified benefits and risks, FDA recommends the classification of HLA, HPA and HNA devices as Class II devices subject to Special Controls. The following regulatory language is proposed:

8XX.XXXX Human Leukocyte, Neutrophil and Platelet antigen and antibody devices

(a) Identification.
Human Leukocyte, Neutrophil and Platelet antigen and antibody devices consist of HLA, HPA and HNA typing and antibody detection devices.

- HLA typing devices are used to determine HLA types, to aid in transfusion or transplantation donor and recipient matching, or to aid in the diagnosis of diseases.

- HLA antibody detection devices are used to detect antibodies to HLA antigens to aid in donor and recipient matching in transfusion or transplantation.

- HPA typing devices are used for the detection of human platelet antigens to aid in donor and recipient matching in blood transfusion or to aid in the diagnosis of diseases.

- HPA antibody detection devices are used to detect autoantibodies and alloantibodies against platelet glycoproteins to aid in donor and recipient matching in blood transfusion, or to aid in the diagnosis of diseases.

- HNA typing devices are used for the detection of human neutrophil antigens to aid in donor and recipient matching in blood transfusion or to aid in the diagnosis of diseases.

- HNA antibody detection devices are used to detect autoantibodies and alloantibodies against neutrophil antigens to aid in donor and recipient matching in blood transfusion or to aid in the diagnosis of diseases.

(b) Classification. Class II (Special Controls).

HLA, HPA and HNA typing devices must comply with the following Special Controls:

1) Premarket submissions must include detailed documentation of the following information:
    a. Device accuracy study using well-characterized samples representing as many targets as possible.

b. Precision studies to evaluate possible sources of variation that may affect test results
c. Comparison studies to evaluate the device's performance compared to a predicate.
d. Specific information that address or mitigate risks associated with false positive antibody reactivity e.g., reactivity with denatured/cryptic epitopes, if applicable.
e. Description of how the assay cut-off was established and validated as well as supporting data.
f. Documentation for device software, including, but not limited to, software requirement specifications, software design specification, e.g., algorithms, alarms and device limitations; hazard analysis, traceability matrix, verification and validation testing, unresolved anomalies, hardware and software specifications; electromagnetic compatibility and wireless testing.
g. For multiplex assays in which large numbers of probes and/or primers are handled during manufacturing process, premarket submissions should provide the design specifications that are in place to prevent incorrect reactivity assignment.
h. Description of a plan on how to ensure the performance characteristics of the device remain unchanged over time when new HLA alleles are identified, and/or reactivity assignments are changed from the assignments at the time the device was evaluated.

2) The device labeling must include:
   a. A limitation statement that reads, "The results should not be used as the sole basis for making a clinical decision."
   b. A warning that reads "The use of this device as a companion diagnostics has not been established."

9. References

1. Dunn PP: Human leucocyte antigen typing: techniques and technology, a critical appraisal. *Int J Immunogenet* 2011, 38:463-473.
2. Tait BD, Hudson F, Cantwell L, Brewin G, Holdsworth R, Bennett G, Jose M: Review article: Luminex technology for HLA antibody detection in organ transplantation. *Nephrology (Carlton)* 2009, 14:247-254.
3. Hayashi T, Hirayama F: Advances in alloimmune thrombocytopenia: perspectives on current concepts of human platelet antigens, antibody detection strategies, and genotyping. *Blood Transfus* 2015, 13:380-390.
4. Lochowicz AJ, Curtis BR: Clinical applications of platelet antibody and antigen testing. *Laboratory Medicine* 2011, 42:687-692.
5. Lubenko A, Savage J: Antigen capture ELISA for platelet antibody detection: choice of conjugate influences assay result. *Transfus Med* 2000, 10:213-218.
6. Heinzl MW, Schonbacher M, Dauber EM, Panzer S, Mayr WR, Kormoczi GF: Detection of granulocyte-reactive antibodies: a comparison of different methods. *Vox Sang* 2015, 108:287-293.
7. Guidance for Industry and Food and Drug Administration Staff: In Vitro Companion Diagnostic Devices 2014.
8. FDA Final Rule: Leukocyte Typing Serum; Revocation of Additional Standards. August 10, 1982.
9. Blood Product Advisory Committee Transcript. *September 15*: 2000.
10. FDA Guidance for Industry: Recommendations for Premarket Notification (510(k)) Submissions for Nucleic Acid-Based Human Leukocyte Antigen (HLA) Test Kits Used for Matching of Donors and Recipients in Transfusion and Transplantation. 2015.
11. The United Network for Organ Sharing (UNOS) Bylaws, Appendix B, Attachment II..
12. Susal C, Opelz G: Current role of human leukocyte antigen matching in kidney transplantation. *Curr Opin Organ Transplant* 2013, 18:438-444.
13. Schlaf G, Pollok-Kopp B, Altermann WW: Sensitive solid-phase detection of donor-specific antibodies as an aid highly relevant to improving allograft outcomes. *Mol Diagn Ther* 2014, 18:185-201.
14. Fatalities Reported to FDA Following Blood Collection and Transfusion: Annual Summary for Fiscal Year 2015.. https://www.fda.gov/downloads/BiologicsBloodVaccines/SafetyAvailability/ReportaProblem/TransfusionDonationFatalities/UCM518148.pdf
15. AABB: TRALI Risk Reduction Requirements, 29th Edition of Standards for Blood Banks and Transfusion Services.
16. Forest SK, Hod EA: Management of the Platelet Refractory Patient. *Hematol Oncol Clin North Am* 2016, 30:665-677.
17. Kopolovic I, Ostro J, Tsubota H, Lin Y, Cserti-Gazdewich CM, Messner HA, Keir AK, DenHollander N, Dzik WS, Callum J: A systematic review of transfusion-associated graft-versus-host disease. *Blood* 2015, 126:406-414.
18. Howell WM: HLA and disease: guilt by association. *Int J Immunogenet* 2014, 41:1-12.
19. Megiorni F, Pizzuti A: HLA-DQA1 and HLA-DQB1 in Celiac disease predisposition: practical implications of the HLA molecular typing. *J Biomed Sci* 2012, 19:88.
20. Sheehan NJ: HLA-B27: what's new? *Rheumatology (Oxford)* 2010, 49:621-631.

21. Amstutz U, Shear NH, Rieder MJ, Hwang S, Fung V, Nakamura H, Connolly MB, Ito S, Carleton BC: Recommendations for HLA-B*15:02 and HLA-A*31:01 genetic testing to reduce the risk of carbamazepine-induced hypersensitivity reactions. *Epilepsia* 2014, 55:496-506.
22. Yeo SI: HLA-B*5801: utility and cost-effectiveness in the Asia-Pacific Region. *Int J Rheum Dis* 2013, 16:254-257.
23. Illing PT, Vivian JP, Dudek NL, Kostenko L, Chen Z, Bharadwaj M, Miles JJ, Kjer-Nielsen L, Gras S, Williamson NA, et al.: Immune self-reactivity triggered by drug-modified HLA-peptide repertoire. *Nature* 2012, 486:554-558.
24. Caro-Oleas JL, Gonzalez-Escribano MF, Gonzalez-Roncero FM, Acevedo-Calado MJ, Cabello-Chaves V, Gentil-Govantes MA, Nunez-Roldan A: Clinical relevance of HLA donor-specific antibodies detected by single antigen assay in kidney transplantation. *Nephrol. Dial. Transplant* 2012, 27:1231-1238.
25. Konvalinka A, Tinckam K: Utility of HLA Antibody Testing in Kidney Transplantation. *J Am Soc Nephrol* 2015, 26:1489-1502.
26. Carmichael MG, Benavides LC, Holmes JP, Gates JD, Mittendorf EA, Ponniah S, Peoples GE: Results of the first phase 1 clinical trial of the HER-2/neu peptide (GP2) vaccine in disease-free breast cancer patients: United States Military Cancer Institute Clinical Trials Group Study I-04. *Cancer* 2010, 116:292-301.
27. Yadav V, Bourdette DN, Bowen JD, Lynch SG, Mattson D, Preiningerova J, Bever CT, Jr., Simon J, Goldstein A, Burrows GG, et al.: Recombinant T-Cell Receptor Ligand (RTL) for Treatment of Multiple Sclerosis: A Double-Blind, Placebo-Controlled, Phase 1, Dose-Escalation Study. *Autoimmune Dis* 2012, 2012:954739.
28. Winkelhorst D, Murphy MF, Greinacher A, Shehata N, Bakchoul T, Massey E, Baker J, Lieberman L, Tanael S, Hume H, et al.: Antenatal management in fetal and neonatal alloimmune thrombocytopenia: a systematic review. *Blood* 2017, 129:1538-1547.
29. Davoren A, Curtis BR, Aster RH, McFarland JG: Human platelet antigen-specific alloantibodies implicated in 1162 cases of neonatal alloimmune thrombocytopenia. *Transfusion* 2004, 44:1220-1225.
30. Mueller-Eckhardt C, Kiefel V, Grubert A, Kroll H, Weisheit M, Schmidt S, Mueller-Eckhardt G, Santoso S: 348 cases of suspected neonatal alloimmune thrombocytopenia. *Lancet* 1989, 1:363-366.
31. Kiefel V, Konig C, Kroll H, Santoso S: Platelet alloantibodies in transfused patients. *Transfusion* 2001, 41:766-770.
32. Kunicki TJ, Beardsley DS: The alloimmune thrombocytopenias: neonatal alloimmune thrombocytopenic purpura and post-transfusion purpura. *Prog Hemost Thromb* 1989, 9:203-232.
33. Mueller-Eckhardt C: Post-transfusion purpura. *Br J Haematol* 1986, 64:419-424.
34. Lubenow N, Eichler P, Albrecht D, Carlsson LE, Kothmann J, Rossocha WR, Hahn M, Quitmann H, Greinacher A: Very low platelet counts in post-transfusion purpura falsely diagnosed as heparin-induced thrombocytopenia. Report of four cases and review of literature. *Thromb Res* 2000, 100:115-125.
35. Popovsky MA, Moore SB: Diagnostic and pathogenetic considerations in transfusion-related acute lung injury. *Transfusion* 1985, 25:573-577.

36. Popovsky MA: Transfusion-Related Acute Lung Injury: Incidence, Pathogenesis and the Role of Multicomponent Apheresis in Its Prevention. *Transfus Med Hemother* 2008, 35:76-79.
37. Middelburg RA, van der Bom JG: Transfusion-related acute lung injury not a two-hit, but a multicausal model. *Transfusion* 2015, 55:953-960.
38. Reil A, Keller-Stanislawski B, Gunay S, Bux J: Specificities of leucocyte alloantibodies in transfusion-related acute lung injury and results of leucocyte antibody screening of blood donors. *Vox Sang* 2008, 95:313-317.
39. Stroncek DF, Fadeyi E, Adams S: Leukocyte antigen and antibody detection assays: tools for assessing and preventing pulmonary transfusion reactions. *Transfus Med Rev* 2007, 21:273-286.
40. Dale DC: How I manage children with neutropenia. *Br J Haematol* 2017, 178:351-363.