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

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Guidance for Industry

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http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

For questions on the content of this guidance, contact OCOD at the phone numbers or email address listed above.

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Guidance for Industry

This guidance represents the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this as listed on the title page.

I. INTRODUCTION

This guidance provides recommendations to submitters (hereafter referred to as "you") and FDA reviewers in preparing and reviewing premarket notification submissions (hereafter referred to as "510(k) submission" or "510(k)") for human leukocyte antigen (HLA) in vitro diagnostic (IVD) device test kits. This guidance applies specifically to nucleic acid-based HLA test kits used for the matching of donors and recipients in transfusion and transplantation, whether testing is for a single locus or for multiple loci simultaneously, for which the premarket submission to FDA will be a 510(k).

Although this guidance applies specifically to 510(k) submissions for HLA test kits, some of the recommendations in this guidance may also be applicable to human neutrophil antigen (HNA) and human platelet antigen (HPA) test kits. We recommend that you consult the Office of Blood Research and Review (OBRR) in FDA's Center for Biologics Evaluation and Research (CBER) for specific guidance regarding premarket submission requirements for HNA and HPA test kits.

This guidance provides detailed information on the types of studies FDA recommends for validation of HLA test kits submitted as 510(k)s and used for the matching of donors and recipients in transfusion and transplantation. More specifically, the document addresses the types of studies and other information that FDA recommends be used in designing and conducting studies for validation of nucleic acid-based HLA test kits and preparing a 510(k) submission.

This guidance finalizes the draft guidance of the same title dated November 2013 (78 FR 69693, November 20, 2013).

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in FDA's guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

Most cells of the body bear human leukocyte antigens. The role of the HLA system is to allow the human immune system to distinguish the body's own blood, organs and tissues from foreign substances. Clinical complications, such as graft rejection or graft-versus-host disease, may occur if the immune system recognizes transplanted cells, tissues, organs, bone marrow, or a transfused unit of blood or blood components as being foreign on the basis of HLA nonidentity or if transplanted immune system cells react in response to the recipient's HLA.

The science and technology upon which HLA test kits are based has become increasingly complex in recent years. Testing has evolved from serology and cell-based methods with little instrumentation to deoxyribonucleic acid (DNA) based and multiplex assays that use complex instruments and software. HLA is one of the most polymorphic systems found in humans. This high degree of polymorphism and the existence of rare phenotypes found in the HLA system represent significant and unique challenges to develop and design studies that validate the accuracy of these HLA test kits. FDA's expectations for analytical and clinical studies conducted in support of a 510(k) submission focus on identifying potential risks to patients if the kits fail to perform as expected. The performance of these kits is critical for ensuring precise and successful matching between donors and recipients of blood and blood components, cells, tissues, bone marrow, and organs.

III. RECOMMENDATIONS FOR THE PREPARATION OF THE HLA TEST KIT PREMARKET NOTIFICATION (510(k)) SUBMISSION

A. Intended Use

A 510(k) submission must contain, among other things, proposed labels and labeling and advertisements sufficient to describe the device, its intended use, and the directions for its use (21 CFR 807.87(e)). In the case of HLA test kits, the intended use should specify the marker the device is intended to detect.

We recommend that the following statement be included in the intended use statement in your 510(k) submission for HLA test kits:

> "To be used to determine {indicate HLA locus or loci}, to aid in transfusion and transplantation donor and recipient matching."

Please consult CBER for specific guidance regarding HLA test kits that have a different intended use than the one recommended in this section.

B.   Device Design

We recommend that you carefully characterize the design of your HLA test kits. For example, you should describe the following elements in your 510(k) submission, where applicable:

*   Test platform (e.g., flow cytometry, instrumentation for multiplex test systems).
*   Composition and layout in spatially-fixed platforms, including feature (e.g., probe) identity and placement, where applicable.
*   Methods used for attaching the capture or probe material to a solid surface, if applicable.
*   Hybridization conditions, washing procedures, and drying conditions (e.g., temperature, length of time).
*   Assay components such as buffers, enzymes, fluorescent dyes, chemiluminescent reagents, other signaling and signal amplification reagents, instruments, software, etc.
*   Methodology for DNA extraction that you provide or that you recommend for users, and other applicable preanalytical elements. If you do not provide sample preparation reagents in your test kits, you should provide specifications (including justification for these specifications) for assessing the quality of the assay input sample so that users can validate their own sample preparation method and reagents.
*   Range of input sample concentrations that meet performance specifications.
*   Internal controls and external controls, and calibrators that you recommend or provide. Validation of controls and calibrators.
*   Stability and reproducibility of the entire test procedure when used for its intended use.
*   For multiplex tests in which the target molecules will contact a number of different probes, the methods used to mitigate the risk for probe cross-hybridization.
*   The methods used to address the potential for probe cross-contamination.
*   The maximum number of samples that can be simultaneously processed.

We recommend that you describe in detail the test kit's methodology for detecting alleles and genotypes, including a detailed description of algorithms and decision trees, as applicable. You should also briefly outline your risk analysis relating to the test kit's methodology for detecting alleles and genotypes.

We also recommend that you include illustrations or photographs of non-standard equipment or methods because these can be helpful in understanding novel methodologies, including the incorporation of features to minimize potential device failures and user errors.

C.   Performance Studies

Performance studies should demonstrate with a high level of confidence that the test kit performs within the established specifications. You should identify the performance characteristics that are to be assessed and establish validation methods and acceptance criteria.

The following subsections include specific recommendations for accuracy studies, precision studies including reproducibility and repeatability, and clinical comparison studies, including recommendations for the study design, samples, and a description of concordance.

1.   Accuracy Studies

Accuracy studies for molecular detection methods should address all probes and/or primers included in the HLA test kit. However, due to the large number of polymorphisms within the HLA system, we realize that it may be impractical to individually measure the accuracy of each polymorphism that a test kit claims to detect. Therefore, we recommend that the accuracy study, which may be conducted in-house, challenge the performance parameters of the test system as follows:

- The study should use nationally or internationally recognized well-characterized DNA samples (described in this section) that represent the most prevalent HLA alleles, and, if possible, also include rare alleles.
- The sample size for each locus should meet the following criteria: use a sufficient number of well-characterized DNA samples such that you can demonstrate that the one-sided 95% lower confidence limit of overall agreement (i.e., concordance) exceeds 0.95 for each test kit (locus) in the submission. The samples in this study should be tested once and cover a reasonable range of genotypes. Sponsors are encouraged to discuss sample selection with FDA in advance to reach an agreement on this process
- Results should be reported using statistically appropriate practices as outlined in the FDA guidance document entitled, "Guidance for Industry and FDA Staff: Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests," dated March 13, 2007 (Ref. 1). That guidance provides recommendations on the handling of equivocal results, discrepant result resolution and how comparison of the results to non-reference standards and methods should be reported.

Sample Types - We recommend the use of well-characterized DNA samples recognized in the United States (U.S.) or abroad that have been extensively tested using a variety of HLA testing methods, including DNA

sequence-based typing. The term "well-characterized DNA sample" will be used henceforth in this document to describe the abovementioned samples.

Concordance Description - The HLA typing results from your test are concordant with the well-characterized DNA sample if one pair of the reported alleles is the same as the typing results of the well-characterized DNA sample.

2.   Precision Studies including Repeatability and Reproducibility

Precision studies should capture possible sources of variation including within run, run-to-run, day-to-day, operator-to-operator, instrument-to-instrument, site-to-site and lot-to-lot variation. Because within run variability can be captured using a measure of how closely measurements within the same run agree (e.g., percent agreement of results within the same run), we refer to this as the repeatability estimate. The repeatability estimate can be captured using data from an internal study or by combining results collected at multiple sites in a reproducibility study. A reproducibility study should be performed at multiple sites using multiple operators with skill levels similar to those of your intended users. You should also perform testing over several weeks and at different times of the day to maximize detection of potential sources of variability. For reagents recommended to be used with automated instruments, the submission should include testing on three separate instruments, one at each site.

You may choose to combine the repeatability study and the reproducibility study into a single study.

We recommend the following precision study design:

- Three study sites, including at least two external sites, with two operators at each site. If feasible, each operator should perform two runs per day.
- Each sample is run in duplicate (for repeatability), on five nonconsecutive days over 20 days using one lot of the test kit. NOTE: Kit and component lot-to-lot studies, using three different lots, may be conducted in-house using the same panel as the external sites.
- The study outlined in this section should be used on each instrument for which you are seeking clearance.
- If you plan to submit a bundled submission, conduct a precision study for each test kit.
- Operator experience may have an impact on test system performance; therefore, operator training is an important

consideration when designing validation studies. Your precision study protocol should include training requirements for the operators. If applicable, operators should be certified prior to participating in the study and the protocol should include certification acceptance criteria. The training program should reflect the training to be received by operators once the product is marketed.

- In general, complete agreement should be expected. Sponsors should perform an investigation and provide justification if there is disagreement.

Sample Types - Perform precision studies using well-characterized DNA samples (precision panel) that ideally should cover every genotype reported by the device. However, due to the large number of possible HLA alleles that may be detected by your test kit, it may not be possible to cover every genotype in the precision panel; therefore, we recommend the following:

- The precision panel should be blinded and should consist of well-characterized DNA samples representing significant diversity of the detectable alleles represented in the test kit. It is preferable that the panel samples differ from those used in the accuracy study.
- The lowest DNA concentration suggested by the test kit's "Instruction for Use" should be used with all samples in the panel, though additional testing using higher concentrations of DNA can also be added.

Concordance Description - The precision study results are concordant with the well-characterized DNA sample results if one pair of the reported alleles is the same as the typing results of the well-characterized DNA sample. In addition, any uncertainty in the typing assignment (i.e., the list of ambiguities) should be reported and compared between operators, sites, runs, repeats, and days.

3. Clinical Comparison Studies

Clinical comparison studies are performed to evaluate the proposed device's performance in a clinical setting, using random clinical specimens and the assay instructions outlined in the proposed labeling. Clinical comparison studies of investigational IVD devices are subject to the investigational device exemption (IDE) provisions of section 520(g) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 360j(g)), and related implementing regulations. You should consider how FDA regulations at 21 CFR Part 812 (Investigational Device Exemptions) apply to your particular study, and also refer to 21 CFR Part 50 (Informed Consent) and

21 CFR Part 56 (Institutional Review Boards) for other applicable requirements.

Informed Consent

For studies of an investigational device, the definition of a (human) subject includes an individual on whom or on whose specimen an investigational device is used (see 21 CFR 812.3(p)). Because section 520(g)(3)(D) of the Federal Food, Drug, and Cosmetic Act and the regulations in 21 CFR Part 812 require informed consent under 21 CFR Part 50 for FDA-regulated human subject research, informed consent must be obtained before specimens can be used, except in limited circumstances as specified in the regulations.[1] Note that the guidance document entitled, "Guidance for Sponsors, Institutional Review Boards, Clinical Investigators and FDA Staff: Guidance on Informed Consent for *In Vitro* Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable," dated April 25, 2006 (Ref. 2) provides that FDA intends to exercise enforcement discretion, under certain circumstances, with regard to the regulations governing the requirement for informed consent when human specimens are used for FDA-regulated IVD device investigations. As described in that guidance document, FDA does not intend to object to the use, without informed consent, of leftover human specimens (i.e., remnants of specimens collected for routine clinical care or analysis that would otherwise have been discarded) in investigations that meet the criteria for exemption from the IDE regulation in 21 CFR 812.2(c)(3), as long as subject privacy is protected by using only specimens that are not individually identifiable.

Note that sponsors must comply with the requirements for institutional review board review, if applicable, as set forth in 21 CFR Part 56 unless they meet the exemption criteria outlined in 21 CFR 56.104 or 56.105.

Study Design for Clinical Comparison Studies

We recommend the following study design:

- The study should include three sites, including at least two external sites of which at least one is in the U.S. The sites should cover different geographic regions and include a representation of major ethnic groups found in the U.S. in order to increase the probability of covering the many HLA genotype variants found in the U.S. population. You can refer to published literature for the U.S. prevalence of HLA alleles. For example, the National Marrow Donor Program (http://www.bethematch.org) provides frequency information for HLA haplotypes found in the U.S. population.
- Your protocol should include training requirements for the operators involved in the studies. If applicable, operators should be certified prior to participating in the study and the protocol should include certification acceptance criteria. The certification/training requirements should reflect those that will be

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[1] 21 CFR 50.23 and 50.24.

implemented once the device is marketed in order to best assess the device performance under actual use conditions.

- Sample size – sufficient number of samples should be tested that would allow one to establish that the one-sided 95% lower confidence limit for the overall agreement with the comparison device exceeds 0.95 for each HLA locus in the submission.
- In your study, you should compare two distinct lots of your product either to a device legally marketed in the U.S. or to results obtained by bi-directional sequencing. Bi-directional sequencing entails sequencing both strands of amplified genomic DNA. Include all test kits that will be described/listed in your submission in the study. The statistical analysis plan should be defined prior to the start of the study and should be consistent with FDA's guidance document entitled, "Guidance for Industry and FDA Staff: Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests," dated March 13, 2007 (Ref. 1).
- The protocol should include evaluation of sample preparation reagents provided with the test kit. If you do not include sample preparation reagents in the test kit, each site should use and validate its own specimen processing procedures and demonstrate that the resulting sample meets the HLA kit manufacturer-supplied specifications.
- Discordant results should be investigated and the results of your investigation should be reported in the submission. However, calculations should be performed on the original results, not the resolved results. Please refer to FDA's guidance document entitled, "Guidance for Industry and FDA Staff: Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests," dated March 13, 2007 (Ref. 1).

Sample Types - Use blinded random samples.

Concordance Description - HLA typing results are concordant with the results from the comparison device if at least one pair of alleles at a specific locus is the same between the two devices, either by allele group or by full allele name. We recommend that you provide an explanation if the list of ambiguities for the predicate device includes a common and well documented (CWD) allele(s) that is not also reported by your device.

NOTE: The term "allele group" refers to those groups of alleles with identical sequences in the exons that encode for the domains of the antigen recognition site (ARS). These HLAs likely present similar or identical peptide binding and immunologic characteristics.

D. Software

If your HLA test kit includes software, you should perform validation as recommended in the FDA guidance document entitled, "General Principles of Software Validation; Final

Guidance for Industry and FDA Staff," dated January 11, 2002 (Ref. 3) in preparing your 510(k) submission.

You should determine the "level of concern" of your software and submit the documentation recommended for that level of concern as discussed in the FDA guidance document entitled, "Guidance for Industry and FDA Staff: Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices," dated May 11, 2005 (Ref. 4). If applicable, you should describe how your software addresses concerns such as background correction, normalization, etc.

If your test kit uses off-the-shelf software, you should follow the recommendations contained in the FDA guidance document entitled, "Guidance for Industry, FDA Reviewers and Compliance on Off-The-Shelf Software Use in Medical Devices" dated September 9, 1999 (Ref. 5).

Algorithms contained in the design of the device are critical to determining the correct genotype and phenotype; therefore, these algorithms should be described in the 510(k) submission.

E.   Validation of Instrumentation

If you provide, or recommend, specific instrumentation for your test kit, whether manufactured by you or by another company, you should include specific information about the instrument(s) in your submission.

If you provide the instrument to the end-user as part of your test kit, your demonstration of substantial equivalence will need to address the inclusion of this instrument in your device. You should address this by submitting instrument validation data in the 510(k). The instrument may be included in the 510(k) for the HLA test kit or the instrument may be submitted as a stand-alone device under a separate 510(k) submission. In the latter case, the HLA test kit and the instrument are reviewed at the same time.

F.   Labeling

Under 21 CFR 807.87(e), the 510(k) submission must include proposed labels, labeling, and advertisements sufficient to describe the device, its intended use, and the directions for its use. Where applicable, photographs or engineering drawings should be supplied.

IVDs, including the HLA test kits covered in this guidance, are subject to labeling requirements under 21 CFR Part 809 (In Vitro Diagnostic Products for Human Use). You must label the product in accordance with these provisions. Furthermore, in addition to the information required in 21 CFR 809.10(b)(2), you should include in the labeling the following statement:

"Should not be used as the sole basis for making a clinical decision."

G.   Additional Considerations

Bundling - You may bundle the test kits for different HLA loci in a single 510(k) submission. However, as is true for any 510(k), you must provide the information required by 21 CFR 807.87. This includes appropriate supporting information for each of the devices or indications for use, as well as data from performance studies under 21 CFR 807.92(b), when applicable. See "Guidance for Industry and FDA Staff: Bundling Multiple Devices or Multiple Indications in a Single Submission," dated June 22, 2007 (Ref. 6).

Changes to the Device - Section 807.81(a)(3) requires a new 510(k) for any change or modification that "could significantly affect" either the safety or the effectiveness of a device. See the FDA guidance document entitled, "Deciding When to Submit a 510(k) for a Change to an Existing Device (K97-1)," dated January 10, 1997 (Ref. 7).

Please note, for the addition of a new test kit locus, submit a traditional 510(k). For example, if your HLA kit was cleared to provide resolution of Class II DRB locus, you need to submit a new 510(k) if you are modifying your kit to include the resolution of Class II DQB locus. We recommend validation testing in accordance with the recommendations described in this guidance. For further information on traditional 510(k)s, see the FDA guidance document entitled "Guidance for Industry and FDA Staff: Format for Traditional and Abbreviated 510(k)s," dated August 12, 2005, and corrected on November 17, 2005 (Ref. 8).

IV.   REFERENCES

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