Regulatory Considerations for HLA Test Kits

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Products Regulated by DRB

- Kits that detect **RED** cell antigens and antibodies
  - Blood Grouping Reagents (BGR)
  - Reagent Red Blood Cells (RRBC)
  - Anti-Human Globulin (AHG)

- Kits that detect **WHITE** cell antigens, genotypes and antibodies
  - Human Leukocyte Antigen (HLA) and Antibodies
  - Human Neutrophil Antigen (HNA) and Antibodies
  - Human Platelet Antigen (HPA) and Antibodies

Products Regulated by DRB (2)

- Fetal Maternal Hemorrhage kits (FMH)
- Hemoglobin and Hematocrit measuring devices
- Blood Warmers
- Blood Establishment Computer Software (BECS) and Software contained in medical devices

HLA Background

- The human leukocyte antigen (HLA) system is the name of the major histocompatibility complex (MHC) in humans. These histocompatibility molecules are glycoproteins expressed at the surface of almost all cells.

- Each individual carries a particular combination of proteins in the HLA set. There are different types of HLA -- A, B, C, DP, DQ and DR. We all have 2 HLA molecules of each type, inherited from either parent. Therefore, the final HLA combination serves as a molecular identifier for an individual. The immune system of the body uses the HLA combination to identify cells in the body as their own.

HLA Background (2)

- Human leukocyte antigen (HLA) typing is used to match patients and donors for bone marrow, cord blood, or solid organ transplants.
- Testing has evolved from serology and cell-based methods with little instrumentation to DNA based and multiplex assays that use complex instruments and software.
- It is challenging to develop and design clinical studies that test the accuracy of these kits due to the high degree of polymorphism in HLA genes and the very low prevalence of rare phenotypes found in the HLA system.

HLA Background (3)

- FDA's expectations for clinical studies in support of approval of these kits focus on identifying potential risks to patients if the kits fail to perform as expected.
- The sensitivity and specificity of these kits are critical for ensuring precise and successful matching between patients and donors in transfusion and transplant medicine.
- HLA-typing has also become important in personalized medicine for determining an individual's risk for certain diseases and drug interactions.

Applicable Regulations, Guidance, and Standards

- This presentation will focus on the following guidance:

  "Pharmacogenetic Tests and Genetic Tests for Heritable Markers, June 19, 2007".

- See last 5 pages for a complete list of applicable reference documents.

Applicable Regulations, Guidance. and Standards (2)

- Most of the HLA submissions are traditional 510(k)s (21 CFR Part 807 – Subpart E)

- Some of these devices may require submission of a PMA (ex. - companion diagnostics) based on intended use claims. Sponsors should consult DRB for determination of submission type. (21 CFR Part 814).

Heritable Marker Guidance Applicability to HLA Test Kits

- Provides recommendations to sponsors and FDA reviewers in preparing and reviewing submissions for pharmacogenetic and other human genetic tests, whether testing is for single markers or for multiple markers simultaneously (multiplex tests).

- Recommends a basic framework for the types of data and regulatory issues that should be addressed in a HLA submission.

Heritable Marker Guidance Applicability to HLA Test Kits (2)

- This presentation will specifically focus on HLA typing kits, but is also applicable to HLA antibody, neutrophil (HNA), and platelet (HPA) antigen and antibody test kits, and kits that detect human erythrocyte antigens (HEA) by molecular methods.

Intended Use

- An application for premarket approval or clearance of a device must include a statement of the intended use of the device. 21 CFR 807.92(a)(5), 814.20(b)(3)(i).

- The intended use of the device for which approval or clearance is sought should specify the marker the device is intended to measure, the clinical purpose of measuring the marker, and the populations to which the device is targeted.

Device Design

An application for premarket approval or clearance of a device must include information on the design of the device. 21 CFR 807.92(a)(4), 814.20(b)(3)(ii), 814.20(b)(4). For example:

- Test platform (e.g., flow cytometry, instrumentation for clinical multiplex test systems).
- Methods used in attaching the probe material to a solid surface.
- Sequence or identity of oligonucleotides, primers, probes, or other capture elements.
- Hybridization conditions, washing procedures, and drying conditions (e.g., temperature, length of time).

Device Design (2)

- Assay components such as buffers, enzymes, fluorescent dyes, instruments, software, etc.
- Specificity of probes and primers for locus of interest;
- Methodology for DNA extraction that you provide or that you recommend for users.
- Sample types.
- Range of input sample concentrations that meet performance specifications.
- Internal controls and external controls that you recommend or provide.
- For multiplex tests that utilize many probes, the methods used to address the potential for probe cross-contamination.

Analytical Studies

Provide analytical studies that evaluate the following performance characteristics:

- General analytical performance considerations
- Sample characterization and specifications
- Effect of excess sample and limited sample
- Controls and Calibrators
- Analytical Specificity
- Assay Conditions
- Sample Carryover and cross-hybridization
- Limiting factors

Analytical Studies (2)

General analytical performance considerations:

- Demonstrate the device's ability to accurately and reproducibly differentiate genotypes and alleles using both the lowest and highest nucleic acid input concentrations recommended in product labeling.
- When fresh samples for rare alleles, genotypes, or mutations are scarce, we will consider the use of archived or retrospective samples.
- Demonstrate that your assay can distinguish between hetero- and homozygotes (HEA)

Analytical Studies (3)

Sample Characterization and Specifications:

- If you intend to provide reagents for specimen processing - demonstrate that the sample preparation method consistently provides quality nucleic acid samples that yield reproducible test results for each specimen type.

- If you do not intend to provide sample preparation reagents - provide specifications in package insert for assessing the quality of the DNA sample. You should provide justification for these specifications in the submission. Conduct studies using at least three commonly used DNA extraction methods using each sample type listed in the package insert. The results from these studies should support the DNA sample specifications stated in the package insert.

Analytical Studies (4)

Sample Characterization and Specifications:

- Perform DNA extraction studies with all sample types and anticoagulants recommended in the package insert. The studies should include samples tested at the end of their dating period. Examples

  Whole Blood/EDTA, Whole Blood/ACD, Buccal Swabs, Buffy Coat.

- Characterize sample stability and validate your storage and hold time recommendations. The studies should include samples tested at the end of their dating period. Examples:

  "DNA can be used immediately after isolation or stored at –20ºC for up to 1 year"

  "The amplified product can be stored up to one week at 2-8ºC prior to use. For longer storage, store at –20ºC up to one month until ready to assay. The amplified product can only be frozen and thawed once"

Analytical Studies (5)

Effect of excess sample and limited sample:

- You should investigate the range of nucleic acid sample concentrations that reproducibly yield acceptable results.
- Determine the minimum amount of DNA sample that provides acceptable performance and approximate the amount of patient specimen needed to generate this minimum amount of DNA sample.

Analytical Studies (6)

Controls and calibrators

- If you do not provide external controls or calibrators, you should indicate commercial availability of these materials or describe a method that users can follow to prepare them.
- Controls should contain nucleic acid levels at the low end of the input concentrations recommended in product labeling in order to adequately stress the system.

Analytical Studies (7)

Analytical specificity (interference and cross-reactivity studies):

- Potential inhibitors present in patient specimens may not be efficiently removed by sample preparation procedures and may even interfere with sample preparation itself.
- Examine potential interfering substances commonly present in the indicated patient specimens for their effects on sample preparation and assay performance.
- Potential interference of residual extraction reagents for isolation of DNA.
- Consult “Interference Testing in Clinical Chemistry;” Approved Guideline, Clinical Laboratory and Standards Institute (CLSI), EP-7A.

Analytical Studies (8)

Assay conditions:

- Verify hybridization conditions (for thermocycling, cycling conditions), concentration of reactants, and control of non-specific activity.
- In the case of multiplex tests, you should examine and describe optimization of multiple simultaneous target detection.
- When thermocycling is used, you should verify optimization, specificity, and robustness of amplification

Analytical Studies (9)

Potential for sample carryover and cross-hybridization

- We recommend that you assess the potential for sample carryover and cross hybridization, and that you provide instructions in your labeling for preventing carryover and reducing or eliminating cross-hybridization.

Analytical Studies (10)

Limiting factors of the device:

- You should describe any known limitations of the device.
- Examples:
  - The device does not measure all possible alleles, genotypes, or mutations, or when the range of alleles, genotypes or mutations is not known.
  - “Beads must be prewarmed”,
  - “Do not mix components from other kits and other lots”
  - “Homozygous results should be verified by another HLA method”

Instrumentation

- If an instrument is considered as part of the HLA kit system it can be submitted under the 510(k) of the kit. If the instrument is to be used with other systems it should be submitted under a separate 510(k).
- You should provide specifications in your labeling for any generic instrument needed to run the test, so that users may select an instrument that is suitable for their purposes. Base your recommendations on performance testing of various instruments with your device.
- If you provide, or recommend, specific instrumentation for your device, whether manufactured by you or by another company, you should include specific information about the instrument(s) in your submission, and you should perform studies using this instrument.

Instrumentation (2)

- Assure that any changes made to the recommended instrument (by you or the manufacturer) are tracked and evaluated to determine whether there is any effect on assay performance, in accordance with the quality system regulation (21 CFR Part 820).

- If changes in the recommended instrumentation introduce new or different assay performance issues, you will be responsible for validation of your device under the changed conditions and you should determine whether you need to submit this information to FDA as a change to your device.

Precision Studies

- Closeness of agreement between independent test results obtained under stipulated conditions.
- Repeatability conditions – Independent test results are obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time (same day, site, operator, instrument, and lot)
- Reproducibility conditions – Test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment (between runs, days, sites, operators, instruments, and lots).

Precision Studies (2)

- Precision panels - evaluate the lowest and highest nucleic acid input concentrations recommended in product labeling.
- Three or more sites.
- Multiple operators with skill levels the same as those of your intended users should perform the test, preferably using multiple lots of devices and reagents.
- Perform testing over several weeks and at different times of the day to maximize detection of potential sources of variability.

Precision Studies (3)

- If you do not include or recommend a specific instrument with the kit, each site should use its own instrument during testing.
- Consult “Guidance for Industry and FDA Staff – Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Test”.

Comparison Studies

Comparison to a Reference Method

- We recommend that you validate your assay by performing studies that compare results obtained with your device to those obtained with bidirectional sequencing. Bidirectional sequencing is considered the reference method for sequence analysis (sometimes referred to as the “gold standard”).
- You may choose to conduct comparison studies with a predicate device. You should generally report results as positive percent agreement and negative percent agreement. For HLA kits, due to the large number of alleles, report results as total percent agreement.
- While comparison to another device can be useful, FDA believes that the best measure of test performance will come from comparison to bidirectional sequencing.

Comparison Studies (2)

Comparison to a Reference Method

- Evaluation of sample preparation reagents if provided with the kit.
- If you do not include sample preparation reagents in the test kit, each site should use and validate its own specimen processing procedures.
- Three or more sites.
- Operators with skill levels the same as those of your intended users should perform the test, preferably using multiple lots of devices and reagents.

Comparison Studies (3)

- Resolution of Comparison Discrepancies - FDA does not recommend the use of resolved results in calculations of device performance unless unbiased statistical techniques can be used. See the guidance, “Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests; Guidance for Industry and FDA Staff”

- Failure Rates - You should identify incorrect results obtained due to device failure. You should also provide estimates of expected failure rates (e.g., when result is “no call” due to device failure or sample inadequacy).

Labeling

- 21 CFR Part 809.10
- Directions for Use - You should provide clear instructions that delineate the technological features of the specific device. Devices incorporating nucleic acid amplification should provide sample work-flow recommendations in the labeling.

Labeling (2)

- Quality Control - Include a description of quality control measures that the laboratory should follow to help ensure proper device performance.
- Interpretations and Precautions - Provide the key for interpretations of results and specify the language to be used in reporting results. You should provide a reference for the nomenclature system that you are using to describe alleles, genotypes, and mutations.
- Address the limitations of your device with statements in the labeling.

Labeling (3)

- Stability - You must include information on the reagent shelf life. (See 21 CFR 809.10(a)(5) and 809.10(a)(6) regarding labeling; 809.10(b)(5)(i) regarding the package insert and 814.20(b)(6)(i) for premarket approval applications.)

- Performance - Device performance in comparison to the reference method. Useful formats include 2 x 2 tables, sensitivity and specificity, percent agreement, etc. You should calculate the sensitivity and specificity or percent agreement with their respective confidence intervals using all tested samples. The number of failed (invalid) assays by your device the sample should be reported in your performance characteristics.

Bundling

- You generally may bundle in a single submission multiple analytes or instruments when the same analytical and clinical data can be used for all the analytes/instruments referenced. (Example – HLA-A, HLA-B, HLA-C, etc).
- Consult “Bundling Multiple Devices or Multiple Indications in a Single Submission, June 27, 2007”.

Relevant Guidance and Standards List

- Pharmacogenetic Tests and Genetic Tests for Heritable Markers, Guidance for Industry and FDA Staff, June 19, 2007.
- In Vitro Diagnostic (IVD) Device Studies – Frequently Asked Questions
- Guidance for Industry and FDA Staff – Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Test

Relevant Guidance and Standards List (2)

- Guidance for Industry and FDA Staff : Commercially Distributed Analyte Specific Reagents (ASRs): Frequently Asked Questions
- Use of Symbols on Labels and in Labeling of In Vitro Diagnostic Devices Intended for Professional Use
- Guidance for Industry and FDA Staff – 510(k) Device Modifications: Deciding When to Submit a 510(k) for a Change to an Existing Device – draft guidance, July 27, 2011

Relevant Guidance and Standards List (3)

- Draft Guidance for Industry and Food and Drug Administration Staff - In Vitro Companion Diagnostic Devices, July 14, 2011
- Guidance for Industry and FDA Staff - Class II Special Controls Guidance Document: Instrumentation for Clinical Multiplex Test Systems, March 10, 2005
- Draft Guidance for Industry and FDA Staff - Commercially Distributed In Vitro Diagnostic Products Labeled for Research Use Only or Investigational Use Only: Frequently Asked Questions – June 01, 2011

Relevant Guidance and Standards List (4)

- Guidance for Industry and FDA Staff – Format for Traditional and Abbreviated 510(k)s, August 12, 2005
- CLSI document - EP07-A2 Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition
- CLSI document - EP12-A2 User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline- Second

Relevant Guidance and Standards List (5)

- Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices - Guidance for Industry and FDA Staff
- General Principles of Software Validation; Final Guidance for Industry and FDA Staff
- Guidance for Off-the-Shelf Software Use in Medical Devices; Final
- Guidance for Industry - Cybersecurity for Networked Medical Devices Containing Off-the-Shelf (OTS) Software
- Guidance for Industry - Computerized Systems Used in Clinical Investigations

THANK YOU