Licensed Immunohematology Products and Associated Instrumentation Part 2

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Outline

- Labeling
- Performance Studies
  - Accuracy Study
  - Real Time Stability Study
  - Transport Stability Study
  - Post Approval Stability Studies
  - Interference Study
  - Sample Type Study
  - Precision Study
  - Comparison Study

LABELING

Labeling

- 21 CFR Part 801 - General labeling requirements for medical devices

- 21 CFR 809.10 - Labeling requirements for IVD products (Immediate container, outer packaging, package insert)

- 21 CFR Part 830.20 - Unique Device Identification (UDI)– general provisions, FDA accreditation of an issuing agency, UDI database. Identify medical devices through their distribution and use

Labeling

- 21 CFR Parts 610.60 through 610.68 - General labeling requirements for biologic products

- 21 CFR 660.28 (BGR), 660.35 (RRBC), and 660.55 (AHG) - Product specific labeling requirements

Labeling

Validate all labeling claims. For example:

- Detection of rare phenotypes
- Intended Use - Donors, patients
- Testing procedure - time and incubation temperature ranges
  - Positive samples – low end of time range
  - Negative samples - high end of time range
- Sample types and sample storage

Labeling

Automated Methods:

- Instrument User Manual
  - Identify reagent manufacturer and applicable reagents
- Reagent Package Insert
  - List instrument(s)
  - Refer to User Manual for operating instructions

Labeling

- Performance Data – include result tables and explanations of discordant results

- Description of expected results:
  - Address all test methods, include expected reaction grades
    - Example: hemagglutination versus adherence for solid phase
  - Photos – important for visual inspection requirements

- User Manual - end user access (hard copy or e-copy or both)

- Procedures provided to end user in addition to the User Manual or package insert – labeling review by FDA

PERFORMANCE STUDIES

General Considerations

- Insufficient sample size
  - Can occur with rare phenotypes, positive antibody screens, antibody identification, positive DAT samples, incompatible crossmatches
  - Affects original BLAs, supplements, lot release testing, stability testing, etc.
  - May negatively affects statistical analysis results
  - Identify problem early in the design and development phase
    - Due diligence
    - Plan to stockpile well-characterized samples and contrived samples

- Definitions:
  - Well-characterized samples - Samples that have been extensively tested using a variety of immunohematology testing methods; minimum of two cell lines
  - Contrived samples - Samples that are prepared or designed to express pre-determined attributes

General Considerations

- Comparison study should not be used to validate all the claims made in the labeling
  - Perform in-house **prospective** validation studies
  - Identify product characteristics prior to performing external comparison studies
  - Prospective validation studies provide information necessary for the design of the comparison study (Sample types, time range for incubation, rare phenotype claims, etc..)

Accuracy Study

- Determine the measurement of agreement between the expected value and the investigational device value

- Early in the design and development process - perform feasibility studies using well-characterized and/or contrived samples

- Perform prospective internal accuracy study using well-characterized and/or contrived samples
  - For least burdensome approach – not necessary if have adequate sample size in the clinical comparison study
    - Example – Anti-A, Anti-B versus Anti-e (negative samples), Anti-C^w (positive samples)
  - Expect 100 percent agreement to expected results – provide explanation for discordant results

Stability Studies

- Demonstrate that the product can maintain its performance characteristics over a defined time interval and within defined storage conditions

- Applicable to both FFMU products and final IVD products

- Three main types:
  - Real-time stability studies
  - Transport stability studies
  - Post approval stability studies

- Pre-defined acceptance criteria - out-of-specification results investigated and explained

Real-time Stability Study

- Three conformance lots
- Use container closure system included in the submission
- For BGR and AHG: Submit a minimum of 25% of the data
  - For example, 6 months of data for a proposed 2 year expiry
- For RRBCs: Short shelf life - submit 100% of the stability data

Real-time Stability Study

- Use test methods in the labeling
- Describe in-house reference material
- Describe testing intervals and study duration
- Testing should extend beyond the proposed shelf-life
- In-use stability – challenge the actual routine use of the IVD in the user environment
  - Example: stability of IVD after opening vial and remaining at room temperature for one or two work shifts

Real-time Stability Study

- Microbiology testing time points – time zero and end of expiry
- For automated methods: On-board stability - the maximum length of time IVDs can be loaded onto an instrument and still perform according to specifications
- Submit additional stability data as it becomes available during the review process

Real-time Stability Study

- For BGR and AHG
  - Potency and specificity testing - include phenotypes of the RBCs used in the study
  - Test results should meet the potency requirements outlined in 21 CFR 660.25 (BGR) and 660.54 (AHG)
  - Reduce unanticipated potency titer variability between the testing time points - control variations in pipetting technique, donor red blood cells, and incubation times

- For RRBC
  - Check for hemolysis
  - Perform direct antiglobulin test
  - Ensure limit of detection is not reduced over time

Transport Stability Study

- Test the transport conditions that will be experienced between the time of manufacture and delivery to end user
  - Determine effect of transport conditions on shelf-life using stability study testing

- Actual transport study - difficult to maintain and/or control environmental conditions

- Transport simulation study – preferred method
  - Challenge at extreme conditions that may occur during shipping and handling of the product
    - High and low conditions for temperature and humidity
    - Drop and vibrations testing

Transport Stability Study

- Same testing timepoints, stability indicating tests, and acceptance criteria as those in the real-time study. If bundled submission – may be possible to use the family or matrix approach
  - Provide justification for the proposed transport study design

- Include all packaging configurations, for example: single packs, ten packs

Post Approval Stability Studies

- Compliance Policy Guide 280.100 – Stability Requirements for Licensed IVD Products – Post approval stability studies performed if:
  - Required as a condition of approval of the license
  - Due to changes in manufacturing or formulation
  - Part of a corrective/preventive action plan

- Stability testing time point failures – report to FDA

Interference Study

- Consider substances that are likely to be present in patient and donor samples that may have the potential to interfere with the test

- Clinical and Laboratory Standards Institute (CLSI) document entitled *EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline-Second Edition*

Interference Study

- Common sample abnormalities such as hemolysis, icterus, lipemia, Wharton's Jelly in cord blood
- Anticoagulants, additive solutions, and preservatives
- Substances that contact specimens – serum separator devices, specimen collection containers and their stoppers
- Limitations and warnings section of package insert
- Comparison study - may provide additional information

Sample Type Study

- Demonstrate that the reagent is not affected by the recommended anticoagulants and sample age listed in the product labeling
- Include all sample types, specimen collection limitations, and sample storage conditions listed in the package insert
- Expect 100% concordance with expected results (well-characterized and contrived samples)

Precision Study

- Demonstrate that the test reagent generates repeatable and reproducible results using a panel of well characterized and contrived samples
- The study should capture all 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

Precision Study

- Test method listed in the Package Insert
- Precision panel samples cover each test listed in the submission – e.g.; Anti-A, Anti-B, Anti-D, antibody screen
- The lot-to-lot study may be performed in-house
  - Use same panel as precision study performed in external sites
- Precision Study Design Example:
  - Tested at three sites (two external sites) by two operators at each site, with each operator performing two runs per day, on five nonconsecutive days, over a 20-day period
  - Each sample is run in duplicate (for repeatability)
  - Lot-to-lot study performed in-house using three lots

Precision Study

Data analysis:

- Acceptance Criteria: 100% agreement between the different sources of variation
- Agreement results should be summarized for each precision panel member separately
- Investigate and provide justification if there is disagreement

Comparison Study

- The Comparison Study evaluates the performance of the investigational reagent compared to a US licensed reagent
- BGRs, AHG, and RRBC are exempt from the IND requirements – 21 CFR Part 312.2(b)2(ii)
- May use de-identified leftover samples

Comparison Study

- Supplement de-identified leftover clinical specimens with well-characterized and contrived samples for the following:
  - Rare phenotypes
  - Positive direct antiglobulin samples (include weak samples)
  - Positive antibody screening samples (include weak samples)
  - Antibody identification samples (include weak samples)
  - Incompatible crossmatch tests (include weak samples)
- Description of the methods used to determine the samples are well-characterized, description of contrived samples

Comparison Study

- Three external sites - Intended Use will determine site selections
  - For donor testing only
  - For donors and patients

- For BGRs, the sites should cover different geographic regions and include a representation of major ethnic groups found in the US – provide summary table

- Compare two distinct lots of the investigational reagent to FDA licensed products
  - If no FDA licensed reagent is available, discuss acceptable alternatives with FDA

Comparison Study

- Include examples of sample types identified in the labeling and validated in a prospective study - provide a summary
- Test the study samples by all test methods and test conditions in the labeling
- Study samples should include patients with various conditions and diseases, neonates, and older patients – provide a summary

Comparison Study – Data Analysis

Recommended Acceptance Criteria:

- **Antigen phenotyping**: the lower bound of the one-sided 95% confidence intervals for the positive percent agreement and the negative percent agreement with the comparator reagent should exceed 99%

- **Antibody screening (non-ABO), antibody identification and direct antiglobulin test:** the lower bound of the one-sided 95% confidence intervals for the positive percent agreement and the negative percent agreement with the comparator reagent should exceed 95% (using random samples)

Comparison Study – Data Analysis

Recommended Acceptance Criteria (cont'd):

- **ABO antibodies (A and B RRBCs):** the lower bound of the one-sided 95% confidence intervals for the positive percent agreements and the negative percent agreements with the comparator reagent should exceed 99%

- **Crossmatch (Immediate Spin and Indirect Antiglobulin Testing):** the lower bound of the one-sided 95% confidence intervals for the positive percent agreement and the negative percent agreement with the comparator reagent should exceed 99%

Comparison Study – Data Analysis

Acceptance Criteria (cont'd):

- If the study does not include a sufficient number of positive or negative leftover samples to meet the acceptance criteria may use contrived, and/or well-characterized samples to increase the sample size
  - Compare the result to the expected result
  - Analyze separately from random sample results
  - Expect 100% agreement

Comparison Study – Data Analysis

Refer to FDA's "*Guidance for Industry and FDA Staff: Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests*", *dated March 13, 2007*

Include a 2x2 result table for each reagent comparing investigational test with comparator test or with expected result for well-characterized or contrived samples. Include measures of positive and negative percent agreement and corresponding confidence intervals

Example of 2x2 Table:

Anti-K Results

Anti-K 2044 Samples		Comparator		PPA (Point Estimate)	99.4%
		Positive	Negative	PPA (95% Lower CI)	97.2%
Investigational	Positive	166	3	NPA (Point Estimate)	99.9%
	Negative	0	1875	NPA (95% Lower CI)	99.7%

Comparison Study – Data Analysis

- Provide sample exclusion criteria (example - insufficient sample, sample condition, “no type determined” (NTD) results for automated test method)

- Perform repeat testing only if allowed in labeling (example - indeterminate/equivocal and invalid results)

- Statistical calculations:
  - Performed on the original results if repeat testing is not allowed in the labeling (example – manual tube method)
  - Performed on repeat testing results if allowed in the labeling (example – automated method)

Comparison Study – Data Analysis

- Resolution testing - investigate discordant results using a referee reagent
  - Comparator reagent results are always assumed to be correct
  - Resolution testing not necessary for discordant results with well-characterized and contrived samples – compared to expected results
  - Unlicensed referee reagent – provide package insert in submission
  - Resolution testing results may provide additional information in statistical analysis assessments

Comparison Study – Data Analysis

- Referee lab – ensure test method used in the investigation is equivalent to investigational method and does not use the same reagents as used in the study

  Example:

  - Incompatible crossmatch test - contrived sample
  - Investigational solid phase device result was negative
  - Referee lab also negative using the tube method/LISS crossmatch
  - Two problems:
    - Contrived sample - Original investigational device result should be compared to expected result therefore resolution testing should not have been performed
    - Referee lab method less sensitive than investigational solid phase method

Comparison Study – Data Analysis

- For antibody detection and identification tests, results should be reported at sample level rather than test level (i.e., one result for each sample)

3-Cell Screen Results

3-Cell Screen Result 1789 Samples		Investigational			
		Positive	Negative	PPA (Point Estimate)	64.7%
				PPA (95% Lower CI)	41.9%
Comparator	Positive	11	6	NPA (Point Estimate)	99.7%
	Negative	6	1766	NPA (95% Lower CI)	99.3%

Antibody ID Initial Results (All Sites; Well-characterized Samples)

283 Samples		Expected Results		
		Positive	% Agreement	100.0%
Investigational	Positive	283		
	Negative	0	PPA (95% Lower CI)	98.9%

Comparison Study – Data Analysis

Indeterminate/equivocal results (automated method):

- Do not discard or ignore
- Follow labeling instructions for repeat testing
- Establish equivocal limits
- Helpful if results are provided in table form

Anti-K Equivocal Rates

Percent Equivocals	Comparator	Investigational	P-value
	1/2045 = 0.05%	0/ 2045 = 0.0%	1(not significantly different)

Comparison Study – Data Analysis

Example 1 – Did not meet acceptance criterion due to sample size.

Anti-e Results

Anti-e 2129 Samples		Comparator		PPA (Point Estimate)	100%
		Positive	Negative	PPA (95% Lower CI)	99.9%
Investigational	Positive	2029	0	NPA (Point Estimate)	100%
	Negative	0	100	NPA (95% Lower CI)	97.0%

Assessment: The PPA met the acceptance criterion. The NPA did not meet the acceptance criterion due to the low frequency of e negative samples in the population. The point estimate was at 100.0%.

Comparison Study – Data Analysis

Example 2 – Did not meet acceptance criterion due to sample size and discordant results

Anti-K Results

Anti-K 2044 Samples		Comparator		PPA (Point Estimate)	99.4%
		Positive	Negative	PPA (95% Lower CI)	97.2%
Investigational	Positive	166	3	NPA (Point Estimate)	99.9%
	Negative	0	1875	NPA (95% Lower CI)	99.7%

- **Assessment:** The performance data met the acceptance criterion for NPA but not PPA. This was due to three false positive results with the investigational device and an insufficient positive sample size due to the low frequency of K antigen in the population. However, the referee method agreed with the investigational device results and the point estimate was at 100.0% (after resolution testing)

Comparison Study – Data Analysis

Example 3 – Did not meet acceptance criterion due to incorrect results.

B Cell Results

2944 Samples		Comparator		PPA (Point Estimate)	99.9%
		Positive	Negative	PPA (95% Lower CI)	99.8%
Investigational	Positiv	2516	5	NPA (Point Estimate)	98.8%
	Negativ	1	422	NPA (95% Lower CI)	97.6%

Assessment: The performance data met the acceptance criterion for PPA but did not for NPA. After resolution testing: one false negative result and one false positive result. Although the NPA results did not meet the acceptance criterion the software would indicate "NTD" due to mismatch of forward and reverse typing results

Comparison Study – Data Analysis

Example 4 – Antibody ID

Antibody ID Initial Results (All Sites; Well-characterized Samples)

283 Samples		Expected Results	% Agreement	100.0%
		Positive		
Investigational	Positive	283	PPA (95% Lower CI)	98.9%
	Negative	0		

Assessment: The samples contained Anti-D, -E, -e, -Fy^a, -Jk^a, -K, -C -c. The results obtained on the investigational instrument (IUO) were compared to the expected results. The IUO correctly identified 100% of the antibodies. The PPA was just under the acceptance criterion of 99% due to the sample size

Summary

- Identify and prospectively validate all labeling claims
- Ensure that the validation reports for the performance studies are well-organized, easy to navigate, and contain accurate information
- Anticipate the number of samples needed for testing and identify solutions for insufficient sample sizes during product development
- Provide an assessment of the test results both in the report and in the labeling

Thank you!