Minimum Guidelines for Validation of NGS for HLA

Upon careful consideration, the ASHI ARB has deemed that HLA typing using Next Generation Sequencing (NGS) represents a new assay system; hence laboratories that intend to add NGS technology are required to submit a Validation Packet to their ARB Commissioner. In keeping with the increased complexity of both technical and bioinformatics processes associated with NGS-HLA testing, ARB has developed guidance for minimum requirements for laboratories to use as they prepare for validation. All laboratories submitting validations must continue to follow the Validation Checklist that is published as Appendix I of the ARB Operations Manual (abbreviated version appended to this document). In addition labs are advised to account for and as applicable submit materials outlined below:

For labs using commercially available kits for NGS:

Parallel Testing:

Validation should be performed based upon the specific parameters that are relevant to the platform used. Since the availability of validation data from outside sources influences the extent to which a laboratory must independently validate the method, the laboratories that use a commercially available kit should:

1. Ensure the validation includes all sample types (e.g., blood, buccal swab) the lab routinely uses for clinical testing.
2. Ensure that the performance characteristics that are generally required for all validations (e.g., specificity, reproducibility) are established. Additionally, the laboratory must validate metrics that ensure high quality results specific to NGS. Examples that are consistently used include average coverage and read depth.
3. Ensure that the validation includes testing for the common variants. For HLA typing, this would require testing the HLA types that the lab frequently encounters. Testing heterozygous and homozygous types should be considered.
4. Ensure the data management system is included in validation. This includes file format and provisions for managing the data files that will accumulate over time.
5. Ensure the validation includes sufficient number of samples and no less than 50 samples for all loci. More importantly, the laboratory should validate the range of number of samples routinely run in each run for the given lab. For example, if a lab routinely runs 10-20 samples on each run, the lab should validate the lower and higher end of their sample number range on a run to ensure comparable endpoints.
6. Ensure to validate reproducibility (e.g., inter-assay, intra-assay, and inter-technologist). This can be accomplished with 3 samples tested at least 3 times. For example, inter-assay validation would require testing at least 3 samples in 3 different assay runs. The endpoints for comparison would include typing assignments as well as quality metrics.
7. Ensure that appropriate criteria for acceptance of sequencing runs are determined. This may be accomplished by defaulting to the acceptable factors and parameters set by each manufacturer.
8. The laboratory must have a provision for new data, such as intron sequences and novel alleles.

Blind Parallel Testing: After the validation is completed, the laboratory is prepared to demonstrate proficiency by blind testing of samples. A minimum of 20 blind samples are necessary to fulfill the requirement. Samples may not be shared between two labs that share the same director. Acceptable performance is **80% or greater of the blind samples concordant at all tested loci at least in the 1st and 2nd**

fields. The blind samples may have been sequenced by NGS or Sanger's method or their high resolution result has been obtained by other methods such as SSO and SSP.

Quality testing: ASHI standards located at D.5.2.11 provide important metric driven elements that must be included.

1) **D.5.2.11.1** Sufficient representation of all pertinent allelic specificities of the locus tested in order to evaluate possible allele dropouts. Alleles with consistently poor representation in, sequencing data (drop-out) must be addressed by alternative methods for detection.
2) **D.5.2.11.2** Document and validate the process/method for preparing the enriched sample for sequencing, including **compliance with relevant vendor specifications.**
3) **D.5.2.11.2** When barcodes are incorporated after target enrichment, fidelity of the barcoding method to identify a particular sample needs to be monitored (e.g. by rotating control samples with different barcode sequences).
4) **D.5.2.11.4** Define and document acceptable analytic performance criteria for the sequencing run **incorporating vendor specifications**.
    a. base quality per read position
    b. average read length
    c. average coverage
    d. uniformity of coverage across the length of the targeted region
5) **D.5.2.11.4** Instrument performance measures must include data from internal control samples and/or vendor supplied quality control material**.**
6) **D.5.2.11.8** Independently validate software programs used to generate genotyping information from next generation sequencing data. Ensure that the genotyping algorithms are appropriate for the sequencing strategy used and the error modalities (e.g., homopolymer errors, substitutions) presented by different sequencing chemistries.

Focused inspection is not required, but validation must be approved by the commissioner and co-chair.

For labs developing own reagent for NGS:

New testing systems require validation to demonstrate that the performance characteristics and clinical efficacy are appropriate for the intended use of the test. Appropriate assessment of laboratory testing systems is essential for the high complexity lab. Validation provides the director with an understanding of the strengths, weaknesses, and limitations of testing performed in the laboratory. It is also the foundation for the accreditation process. As such, it is expected from a lab that is developing their own reagents to run sufficient number of samples to optimize their protocol.

Reference:

Volume 76, Issue 12 of Human Immunology, edited by D. Monos and M. Maiers is dedicated to Single-Molecule DNA Sequencing.