# Multi-center Evaluation of HybriType™ for High-Resolution HLA Typing

## Highlights

- The One Lambda™ HybriType™ HLA Plus Typing Flex-96 Kit, RUO (HybriType RUO kit) is a hybrid capture-based target enrichment assay designed to deliver a streamlined workflow, flexible gene content, comprehensive gene coverage, copy number variation (CNV) assessment, and consistent performance across multiple sample types. The Hybritype RUO kit is intended for research use only, not for use in diagnostic procedures.
- Five independent HLA typing centers evaluated the analytical performance of the HybriType RUO Kit, including accuracy, sensitivity, and specificity, using clinical samples previously characterized with other HLA genotyping assays.
- HybriType library preparation and enrichment steps achieved low duplication rates, improving sequencing efficiency by increasing library complexity and the proportion of usable, unique reads.
- Analytical performance demonstrated 100% concordance with a comparator device and minimal allele ambiguity. High specificity was supported by on-target rates exceeding 69%, and high sensitivity was demonstrated by average exon coverage greater than 100x across all samples derived from peripheral blood, buccal swabs, and saliva.

## Introduction

Next-generation sequencing (NGS) technologies for Human Leukocyte Antigen (HLA) typing have evolved significantly over the past decade, driven by user feedback, workflow optimization, and advances in sequencing platforms. PCR-based HLA typing methodologies are widely used reference methods due to their speed, reliability, and low DNA input requirements. However, hybrid capture-based typing assays are gaining momentum due to their tolerance of polymorphisms in primer-binding regions, enhanced performance with lower-quality DNA samples, and reduced susceptibility to PCR-related artifacts. These advantages are enabled using long (100-120 bp) biotinylated probes that capture and enrich target genes independent of specific primer binding sites.

The HybriType RUO Kit was developed to provide an alternative HLA typing solution designed to address limitations associated with existing NGS commercial products. The assay offers a streamlined, flexible, and robust hybrid capture workflow for processing whole blood and buccal swab samples. The HybriType workflow consists of two parts: a) genomic library preparation, and b) hybrid capture-based target enrichment. The workflow has been verified on four Illumina sequencing systems, including the MiSeq i100 Series™, MiSeq™, MiniSeq™, and iSeq 100™. Sequencing output files are imported into TypeStream™ Visual (TSV) software for genotype and phenotype assignments using the appropriate catalog and analysis files.

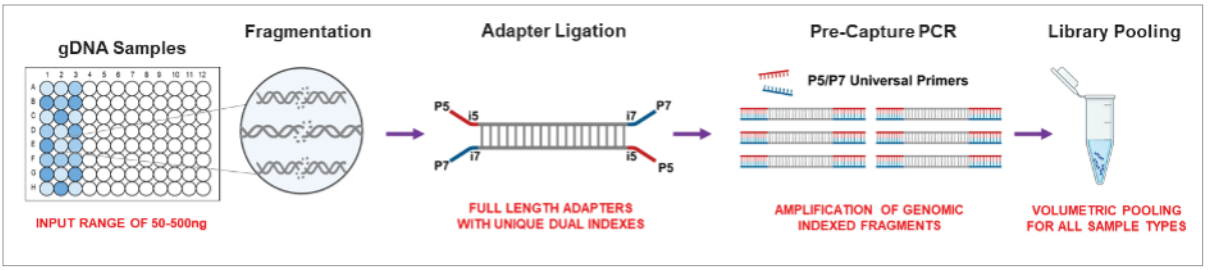
For this multi-center study, five independent HLA typing centers were recruited to evaluate the analytical performance of the HybriType Kit using DNA sample types of their choice that had been previously genotyped with alternative commercial NGS kits. Predefined acceptance criteria were established prior to testing and included HLA typing concordance (accuracy), percentage of on-target reads (specificity), coverage depth in exons, allele balance in exons (sensitivity), and duplication rate across sequencing runs.

## Technical Advantages of the HybriType RUO Kit

### Streamlined Workflow

The HybriType workflow is a two-part system consisting of (A) genomic library preparation and (B) hybrid capture target enrichment steps (Figure 1). The assay follows a standardized protocol across different sample types and includes reagents in a pre-mixed, ready-to-use format to help minimize user error. Short thermocycler incubation steps enhance laboratory efficiency and support faster turnaround times. Technicians experienced in NGS can process 24 samples in less than 5.5 hours, with approximately 2.5 hours of hands-on time.

**A. Genomic Library Preparation | TAT:** 2 hr 6 min **HOT:** 1hr 3 min




**B. Hybrid Capture Target Enrichment | TAT:** 2 hr 58 min **HOT:** 1 hr 4 min

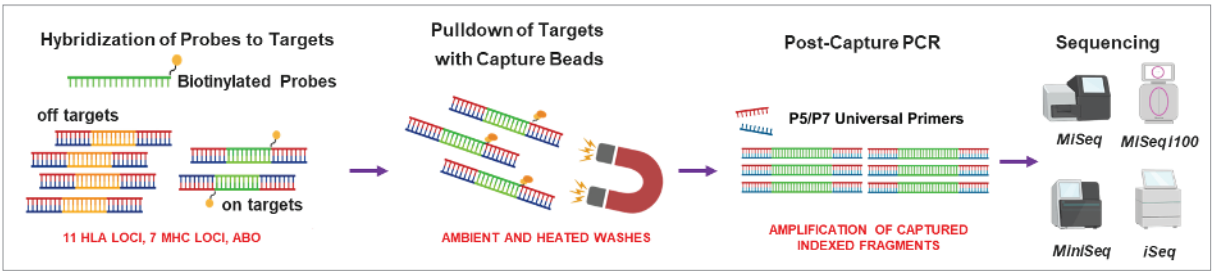



**Figure 1.** Overview of the HybriType workflow (A) Genomic Library Preparation (B) Hybrid Capture Target Enrichment.

## Modular Target Enrichment

The HybriType RUO Kit has flexible probe panel configurations that support routine molecular typing of the 11 classical HLA loci, 7 extended MHC loci, and the ABO gene. Four different probe panel configurations have been evaluated (Table 1). Laboratories can customize gene content by spiking in specific probe panels, enabling optimized data output per test while helping reduce overall cost and maximizing sequencing capacity.

**Table 1.** HybriType offers flexible probe panel configurations to accommodate user needs

| Configuration | 11 HLA Loci | 7 MHC Loci | ABO | Total Gene Content |
|---|---|---|---|---|
| #1 | • | | | 11 Loci |
| #2 | • | • | | 18 Loci |
| #3 | • | • | • | 19 Loci |
| #4 | • | | • | 12 Loci |

## Expanded Gene Coverage

HybriType probe panels span entire gene targets with a high level of redundancy, resulting in reduced ambiguities and higher typing resolution (Table 2). Uncharacterized or complex structural regions, including homopolymer and microsatellite regions, may be excluded from analysis to maintain a high level of specificity and reliable alignment across targeted genes.

**Table 2.** HybriType Gene Content, Coverage and Typing Resolution

**11 Loci Probe Flex Panel**

| Target | Gene Coverage | Resolution |
|---|---|---|
| HLA-A | Full Gene | Field-4 |
| HLA-B | Full Gene | Field-4 |
| HLA-C | Full Gene | Field-4 |
| HLA-DRB1 | Exon 1, Exon 2 through 3'UTR | Field-3 |
| HLA-DRB345 | Exon 1, Exon 2 through 3'UTR | Field-3 |
| HLA-DQA1 | Full Gene | Field-3 |
| HLA-DQB1 | Full Gene | Field-3 |
| HLA-DPA1 | Full Gene | Field-3 |
| HLA-DPB1 | Exon 1, Exon 2 through 3'UTR | Field-3 |

The Hybritype RUO kit is intended for research use only, not for use in diagnostic procedures.

### 7 Extended MHC Loci Probe Flex Panel

| Target | Gene Coverage | Resolution |
|---|---|---|
| HLA-E | Full Gene | Field-4 |
| HLA-F | Full Gene | Field-4 |
| HLA-G | Full Gene | Field-4 |
| HLA-H | Full Gene | Field-4 |
| HLA-J | Full Gene | Field-4 |
| MICA | Exon 1, Exon 2 through 3'UTR | Field-2 |
| MICB | Exon 1, Exon 2 through 3'UTR | Field-2 |

### ABO Probe Flex Panel

| Target | Gene Coverage | Blood Group Phenotypes |
|---|---|---|
| ABO | Exon 1, Exon 2 through 3'UTR | A1, A2, B, A1B, A2B, O |

## Copy Number Variation

The HybriType assay supports discrimination among homozygous, heterozygous, hemizygous, and nullizygous genotypes. The TypeStream™ Visual Software version 3.2 (or higher) evaluates read distribution within and across samples to determine copy number and uses allele balance at variant positions to differentiate homozygous from heterozygous alleles (Figure 2).

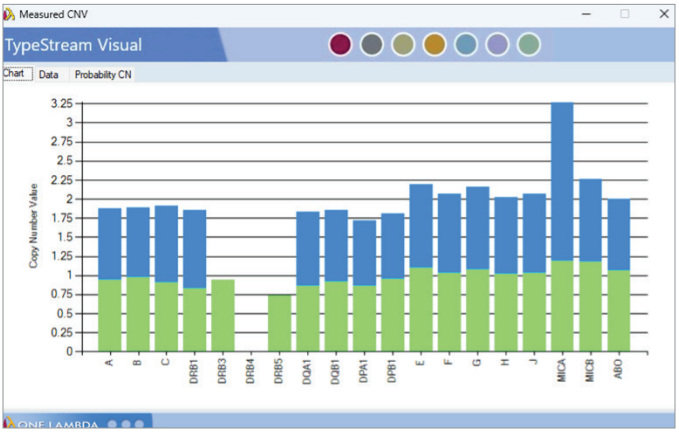



**Figure 2.** Measured copy number variation (CNV) in the TSV software. The green bar represents measured allele 1, and the blue bar represents measured allele 2. The sample shown has two copies for most targets, except for DRB3/4/5 and MICA. DRB3 and DRB5 show one copy each, while MICA shows three copies.

## Compatibility with Illumina Platforms and Kits

The HybriType assays analytical attributes have been tested and verified for use on the Illumina MiSeq i100 Series, MiSeq, MiniSeq, and iSeq 100 sequencing platforms. The recommended batch size ranges from 6 to 96 samples. Table 3 summarizes compatible sequencing kits and maximum sample throughput for 19 loci.

**Table 3.** Sample throughput for 19 Loci HybriType libraries with Illumina sequencers.

| Sequencing platform | Sequencing Kit | Maximum # of Samples |
|---|---|---|
| MiSeq i100 Series | MiSeq i100 Series 25M Reagent Kit (300-cycle) | 96 |
| | MiSeq i100 Series 5M Reagent Kit (300-cycle) | 24 |
| MiSeq | MiSeq Reagent Kit v2 (300-cycle) | 80 |
| | MiSeq Reagent Micro Kit v2 (300-cycle) | 24 |
| | MiSeq Reagent Nano Kit v2 (300-cycle) | 6 |
| MiniSeq | MiniSeq High Output (300-cycle) | 96 |
| | MiniSeq Mid Output (300-cycle) | 48 |
| iSeq 100 | iSeq 100 i1 Reagent v2 (300-cycle) | 24 |

## Materials and Methods

### DNA Samples

Five independent HLA typing centers evaluated the performance of the HybriType RUO Kit using clinical samples that were previously characterized with commercial PCR-based or hybrid capture NGS assays. The majority of DNA samples (~89%) were extracted from peripheral blood, while buccal swab and saliva samples comprised ~11% of the total sample set. Four sites processed batches of 48 samples, and one site processed a batch of 24 samples. Buccal swab and saliva samples generally yielded lower DNA input amounts compared to peripheral blood samples; however, genomic library preparation was successfully completed for all samples. Notably, one buccal sample was prepared using a total DNA input of 20 ng, below the assay's recommended minimum input requirement of 50 ng. All other clinical samples were within the optimal input range for genomic library preparation (Table 4).

**Table 4.** Batch size, sample type, DNA input range, and sequencing platforms used across test sites.

| Test Site | Batch Size | Sample Type | | | DNA Input Range |
|---|---|---|---|---|---|
| | | Blood | Buccal | Saliva | |
| Site #1 | 48 | 37 | 11 | Not Applicable | 20-225ng |
| Site #2 | 48 | 48 | Not Applicable | Not Applicable | 53-230ng |
| Site #3 | 48 | 44 | 4 | Not Applicable | 102-259ng |
| Site #4 | 24* | 23 | Not Applicable | Not Applicable | 61-487ng |
| Site #5 | 48* | 39 | 6 | 2 | 51-171ng |

*A Negative Control (NC) sample was included in the sample batch.

## Genomic Library Preparation, 18 Loci + ABO Target Enrichment, and Sequencing

All clinical DNA samples were processed in accordance with the One Lambda HybriType HLA Plus Typing Flex Kit Application Note (RUO). Briefly, genomic DNA inputs ranging from 20 to 487 ng were enzymatically fragmented, followed by ligation of full-length Unique Dual Index (UDI) adapters, library amplification, and volumetric pooling of all libraries into a single tube. The pooled genomic libraries were enriched for 11 HLA loci, 7 extended MHC loci, and ABO using proprietary capture probes that hybridize to complementary target sequences to enable streptavidin-based pull-down. Off-target sequences were removed through a series of ambient and heated wash steps. Captured libraries were subsequently amplified using a short PCR program prior to sequencing on Illumina platforms. Four HLA typing centers performed sequencing on the MiSeq platform using either the MiSeq v2 Reagent Kit or MiSeq v2 Reagent Micro Kit, while one center used the iSeq 100 platform with the iSeq 100 i1 Reagent v2 kit (300-cycle). Flow cells were loaded at either 60% or 100% sample capacity, depending on reagent availability or individual laboratory preference (Table 5).

**Table 5.** Sequencing instruments used by external sites for HybriType libraries.

| Test Site | Sequencing Platform | # of Samples per Sequencing Run | # of Sequencing Runs | Flow Cell Capacity |
|---|---|---|---|---|
| Site #1 | MiSeq | 48 | 1 | 60% |
| Site #2 | MiSeq | 48 | 1 | 60% |
| Site #3 | iSeq 100 | 24 | 2 | 100% |
| Site #4 | MiSeq | 24 | 1 | 100% |
| Site #5 | MiSeq | 48 | 1 | 60% |

## Data Analysis

FASTQ files were analyzed using TypeStream Visual (TSV) Software version 3.2 (engine version 3.2.0.84; IMGT database version 3.60.0.0) for genotypic assignment of targeted loci and ABO phenotype prediction. The HYB18ABOFX_001_02 catalog file and TSV_HYBRID_CAPTURE_ABO analysis parameters were applied across all analysis sessions. When minimum system requirements were met, the average analysis time was approximately 4.5 minutes per sample. The Copy Number Variant (CNV) tool within TSV was evaluated to differentiate homozygous, hemizygous, and nullizygous genotypes. Emphasis was placed on loci with known copy number variability, including HLA-DRB3/4/5, HLA-H, and MICA, and results were assessed in the context of established linkage disequilibrium patterns.

## Performance Metrics

To assess the analytical performance of the HybriType RUO Kit at external HLA typing centers, a predefined set of performance parameters and acceptance criteria analytical was established prior to testing (Table 6). These metrics, commonly used in the evaluation of commercial NGS assays, were selected to measure accuracy, sensitivity, specificity, and overall robustness. All analytical performance metrics, with the exception of duplication rate, were generated using the TypeStream Visual Software.

**Table 6.** Performance Parameters and Acceptance Criteria for HybriType Kit

| Parameter | Definition | Passing Criteria | Analysis Method/Software |
|---|---|---|---|
| Concordance at Field-3 level for HLA-A, -B, -C, -DQA1, -DQB1, -DPA1, -DPB1, -DRB1/3/4/5 | Accuracy of HLA allele call against the reference typing using 3-Field comparison | ≥ 99.0% | TypeStream™ Visual NGS Analysis Software |
| Concordance at Field-3 level for HLA-E, -F, -G, -H, -J. And Field-2 for MICA and MICB | Accuracy of HLA allele call against the reference typing using 3-Field and 2-Field comparison | ≥ 99.0% | TypeStream™ Visual NGS Analysis Software |
| Concordance of ABO phenotype | Accuracy of ABO phenotype against reference using comarpator device | ≥ 99.0% | TypeStream™ Visual NGS Analysis Software |
| Read Depth for Exons | Average read counts for all targeted exons | ≥ 100x average coverage for 95% of loci tested | TypeStream™ Visual NGS Analysis Software |
| Allele Balance for Exons | The ratio of read counts in exons for allele 1 and allele 2 | ≥ 0.3 average allele balance for 95% of loci tested | TypeStream™ Visual NGS Analysis Software |
| On-Target | The ratio of reads aligned to targeted regions over total number of reads | ≥ 60% | TypeStream™ Visual NGS Analysis Software |
| Insert Size | The length of the DNA fragment between the sequencing adapters | ≥ 400bp | TypeStream™ Visual NGS Analysis Software |
| Duplication Rate | The ratio of the number of duplicates over the number of uniquely mapped reads | ≤ 35% | WinSCP & PuTTY |

## Results

### HLA Typing Concordance for Clinical Samples

HLA typing concordance is one of the most critical performance metrics for NGS-based assays, as it has direct implications in donor-recipient compatibility, which in turn influences graft survival, rejection risk, and overall patient outcomes. Sequencing of 214 clinical samples on the MiSeq and iSeq 100 instruments generated a total of 7,236 allele calls, of which 4,494 had available reference typings. Of these, 4,492 allele calls were concordant with reference typings (Table 7). Participating HLA typing centers provided Field-3 or Field-2 resolution typings for the clinical samples included in this evaluation. Four of the five centers did not provide reference typings for the extended MHC loci, and none provided ABO phenotype data; however, other performance metrics were still evaluated for those loci. The two allele calls initially identified as discrepant fell into two categories: (1) The reference allele was not assigned by the comarpator device and required manual editing to determine the final allele call, and (2) Updates to allele nomenclature in the IMGT reference database resulted in changes to allele names (Table 8). Neither scenario represents a true typing discrepancy. Rather, these differences reflect limitations of the comarpator device and updates in allele nomenclature. After reviewing nomenclature updates and comarpator device limitations, the HybriType RUO Kit demonstrated 100% concordance for all evaluated clinical samples.

**Table 7.** Typing Concordance for Clinical Samples Across Test Sites.

| Test Site | # of Alleles Analyzed | # of Alleles with Known Types | # of Discordant Alleles | % 2- Field or 3-Field |
|---|---|---|---|---|
| Site #1 | 1,622 | 954 | 0 | 100% |
| Site #2 | 1,628 | 862 | 0 | 100% |
| Site #3 | 1,626 | 1,434 | 1 | 99.9% |
| Site #4 | 776 | 408 | 0 | 100% |
| Site #5 | 1,584 | 836 | 1 | 99.9% |
| **Total** | 7,236 | 4,494 | 2 | 99.9% |

**Table 8.** HLA typing discrepancies to reference typings

| Locus | Reference Typing | HybriType Typing | Root Cause for Discrepancy |
|---|---|---|---|
| HLA-A | A*33:03:02 | A*33:03:01 | HybriType allele call is correct. Reference allele type required manual review and editing of the sequence to generate typing due to mismatch in Exon 3 codon 149.3 and high background in Exon 4. HybriType allele call did not show a mismatch to consensus or high background in exons. |
| HLA-C | C*04:09L | C*04:09N | HybriType allele call is correct. Reference allele type was renamed in newer IMGT nomenclature. |

## Ambiguity Resolution Across All Loci

Full gene characterization of HLA loci by NGS is becoming increasingly important in transplantation because it provides high-resolution, allele-level typing and supports accurate detection of rare, null, and structurally complex variants that may influence compatibility in both solid organ and hematopoietic stem cell transplantation. By reducing allele ambiguity, supporting epitope-level risk assessment, and supporting matching precision, full gene sequencing strengthens clinical decision-making and may support improved transplant outcomes.

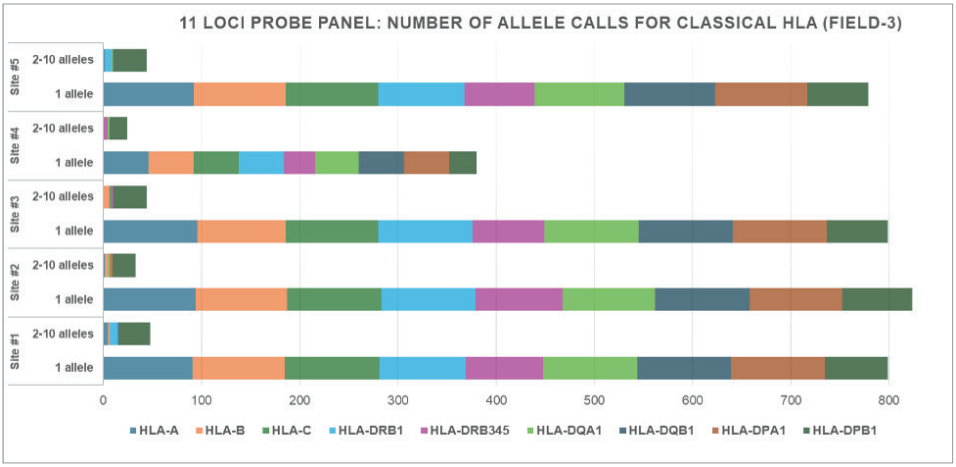
## 11 Loci

The HybriType 11 Loci Probe Flex Panel contains more than 1,600 biotinylated probes targeting exons, introns and untranslated regions across all classical HLA genes. For HLA Class I genes (HLA-A, -B, -C), probes span the full gene sequence, and 4-field typing resolution has been verified. For HLA Class II genes (HLA-DRB1, -DRB3/4/5, -DPA1, -DPB1, -DQA1, -DQB1), probes also span the full gene, with the exception of a portion of intron 1 in DRB1 and DPB1. This region was excluded from the probe design due to limited reference sequence availability and/or structural complexity that could compromise assay specificity. For HLA Class II genes, 3-field typing resolution has been verified. Allele call resolution was assessed at the 3-field level across all sites for the 11 Loci Probe Panel. Of the 3,774 alleles evaluated, 3,581 alleles (95%) yielded a single allele assignment, while 193 alleles (5%) resulted in more than one assignment, but fewer than 10 candidate alleles per case (Figure 3A). These instances represent cis-trans ambiguities, most commonly in DPB1, arising from limitations in phasing across variant positions, a known constraint of short-read sequencing on Illumina platforms. In select cases, intronic coverage within DPB1 contributed to resolution of ambiguities by providing additional phasing information.

## 7 Extended MHC Loci

The HybriType 7 Extended MHC Loci Probe Flex Panel is an optional module targeting non-classical HLA genes in addition to MICA and MICB. When combined with the 11 Loci Panel, this configuration provides expanded genetic information that may support improved transplant risk assessment and enhanced understanding of immune responses (Sayer et al 2025). For non-classical HLA genes, probes span the full gene, and 4-field typing resolution has been verified. For MICA and MICB, probes exclude most of intron 1 but cover the remainder of the gene, and 2-field typing resolution has been verified. Allele resolution for the extended MHC loci was assessed at 3-field for HLA genes and 2-field for MICA and MICB. Of the 2,915 alleles evaluated, 2,717 alleles (93%) yielded a single allele assignment, while 198 alleles (7%) resulted in more than one possible assignment but fewer than 10 candidate alleles per case (Figure 3B). Similar to the 11 Loci Panel, these cases represent cis-trans ambiguities, most commonly observed in MIC genes, due to limited phasing capability inherent to short-read chemistry.

### A. 11 Loci Probe Flex Panel



B. 7 Loci Probe Flex Panel

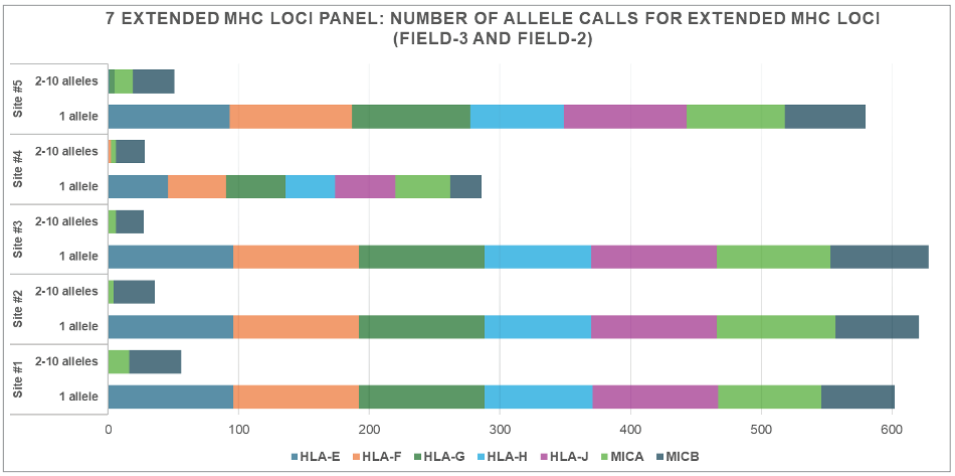
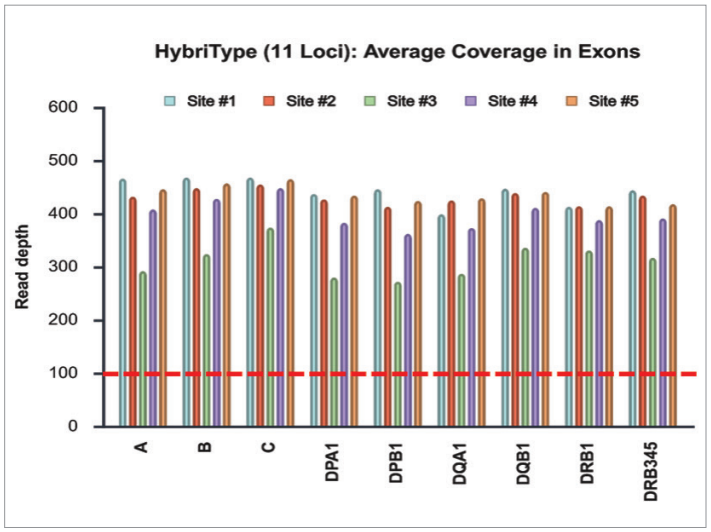



**Figure 3.** Allele typing resolution for the HybriType™ 11 Loci Probe Panel (A) and 7 Extended MHC Probe Panel (B). The number of single allele assignments was determined for classical and non-classical HLA genes at 3-field resolution and MIC genes (MICA and MICB) at 2-field resolution.

## Average Coverage and Allele Balance in Exons

Robust exon coverage and balanced allele representation are essential quality metrics for NGS-based HLA typing assays, as they directly influence typing accuracy, ambiguity resolution, and confidence in genotype assignment. Comprehensive and uniform coverage across exons, particularly within the antigen recognition domain (ARD), supports reliable detection of defining polymorphisms that differentiate HLA alleles. In addition, a proportional representation of allele 1 and allele 2 in heterozygous samples (i.e. allele balance), may reduce the likelihood of technical bias and supports reliable copy number variation (CNV) assessment. Collectively, these metrics are important indicators of assay robustness and are foundational to delivering accurate, high-resolution HLA typing results that support confident clinical decision-making.

The average coverage is defined as the number of sequencing reads aligning to a given position within the targeted locus. Figure 4 illustrates the average exon coverage across all 19 loci evaluated using the HybriType Kit. In all cases, average exon coverage exceeded the predefined acceptance criteria of 100x across all loci tested. MiSeq sequencing runs generated higher-than-expected read outputs. At three of the four MiSeq sites, flow cells were loaded at 60% capacity, resulting in more reads beyond those required for analysis. Because the maximum number of reads used for TypeStream Visual analysis is 300,000 per sample, reads above this threshold were not utilized for typing. In contrast, iSeq 100 runs generated read outputs consistent with expected performance, with flow cells loaded at 100% capacity. As a result, all reads produced by the sequencer were used for typing (<300,000 reads per sample). Although overall coverage at Site #3 was slightly lower compared to MiSeq sites, it remained well within predefined performance specifications.



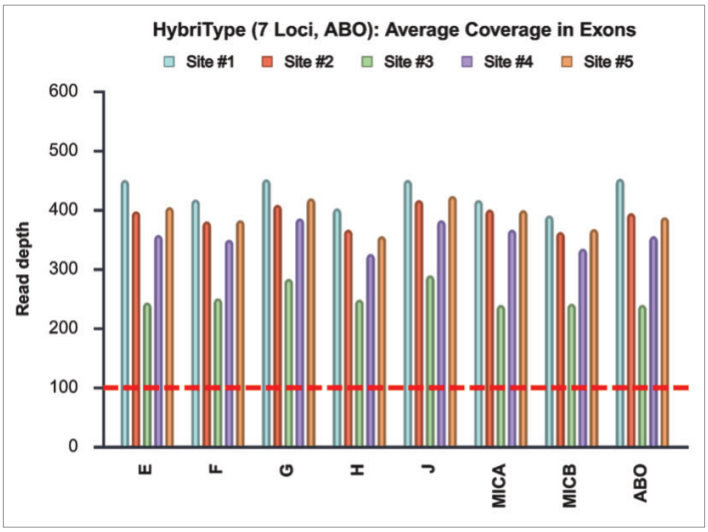




**Figure 4.** Average coverage across all exon regions for the 11 Loci (A), 7 extended MHC loci, and ABO gene (B) targeted by the HybriType assay. All loci showed average coverage above 100x for all sequencing runs with flow cells loaded at 60% to 100% sample capacity.

Allele balance refers to the proportion of sequencing reads supporting each allele (allele 1 and allele 2) in heterozygous samples. Unlike PCR-based typing assays, which may be subject to amplification bias, allelic dropout, or poor coverage uniformity, the HybriType assay demonstrated consistent and stable allele balance across exons for all loci evaluated. At Site #3, various clinical DNA samples that previously showed poor allele balance across multiple loci and allelic dropout of a rare HLA-DRB4 allele when tested with the comparator device were evaluated with the HybriType Kit. These issues were not observed with the HybriType Kit (Figure 5 and Figure 6). Average allele balance in exons exceeded the predefined acceptance criterion of ≥0.3, indicating strong assay sensitivity and high overall data quality.

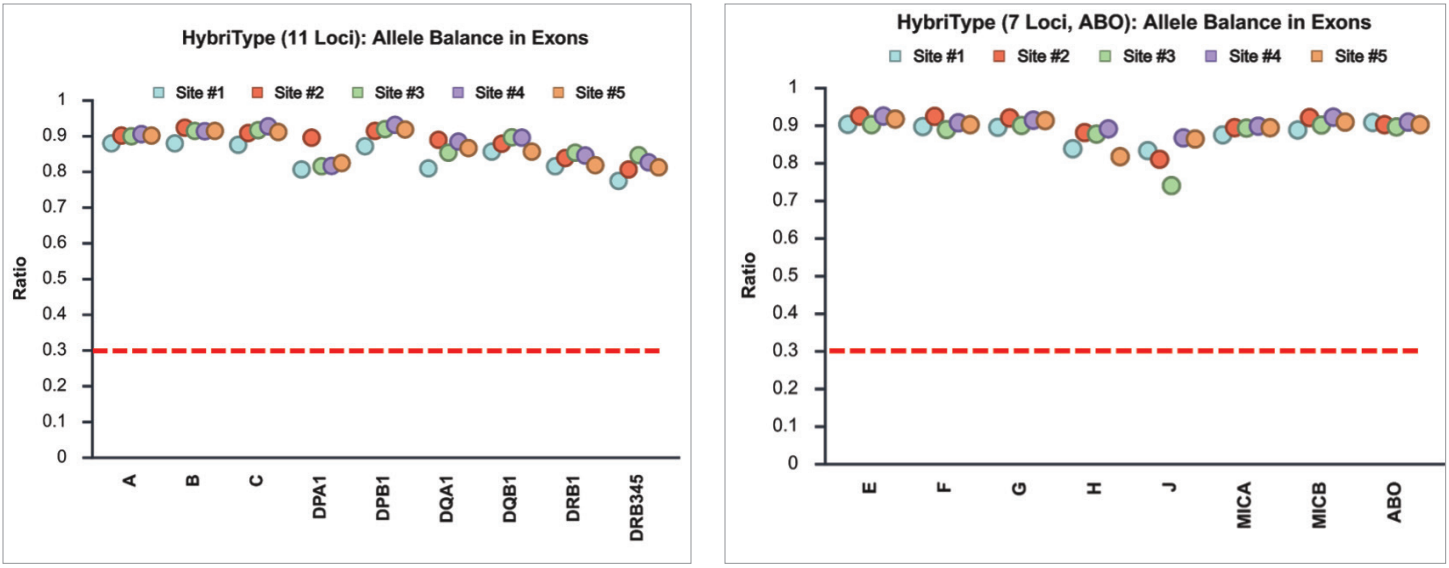



**Figure 5.** Average allele balance in exons for the 11 Loci Panel, 7 Extended MHC Loci Panel, and ABO gene targeted by the HybriType assay. All loci demonstrated average allele balance values above the predefined acceptance threshold of 0.3 across heterozygous samples.

| Locus | Allele 1 | [K/N/I] 1 | Allele 2 | [K/N/I] 2 | Min Coverage | Max Coverage | Coverage |
|---|---|---|---|---|---|---|---|
| DRB1 | DRB1*07:01:01 | [0/0/0] | DRB1*13:02:01 | [0/0/0*] | 212 | 478 | |
| DRB345 | DRB3*03:01:01 | [0/0/0*] | DRB4*03:01N | [0/0/0*] | 239 | 489 | |

**Figure 6.** Detection of DRB4*03:01N allele in a clinical sample with the HybriType Kit.

## On-Target Rate and Insert Size

The on-target rate refers to the percentage of sequencing reads that map to the intended targets defined by the probe panels (11 Loci Probe Panel, 7 Extended Loci Probe Panel, and ABO Panel). High on-target rates reflect effective probe hybridization, optimized wash conditions, and efficient enrichment of intended targets, maximizing usable sequencing output. On-target rates ≥ 60% help ensure that most sequencing reads contribute to HLA typing, while lower on-target rates result in wasted sequencing capacity and reduced coverage for targeted HLA loci.  Across all participant sites, the average on-target rates ranged from 69% to 80%, meeting the predefined acceptance criteria of at least 60% (Figure 7). This resulted in adequate coverage across highly polymorphic loci and increased confidence in genotype assignments.

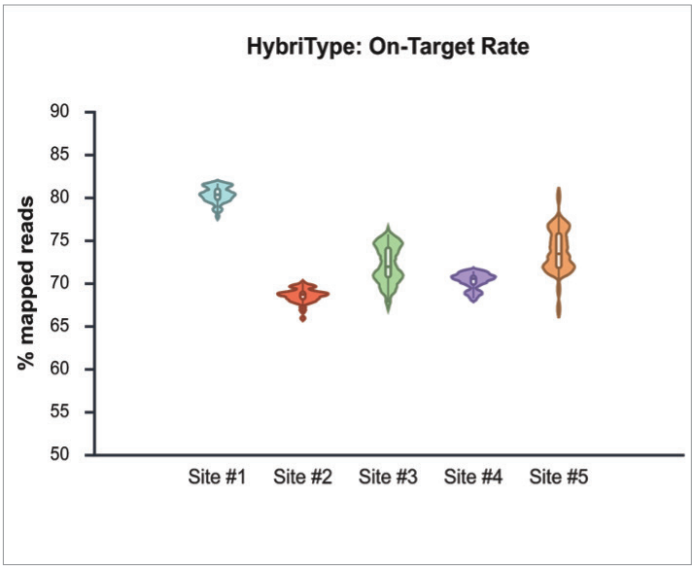



**Figure 7.** Average percentage of on-target reads for 19 loci using the HybriType Kit.

Insert size refers to the length of the DNA fragment between sequencing adapters (i.e. the captured genomic fragment that is sequenced) and reflects the size distribution of enriched library fragments. For Illumina paired-end sequencing systems, larger insert sizes may enhance phasing of variants by increasing the distance spanned by read pairs, thereby reducing cis-trans ambiguities and improving allele resolution. Site #1 exhibited the shortest average insert size (439bp), which included buccal swab samples with lower DNA integrity and lower input amounts (as low as 20 ng). In contrast, Site # 2 showed the longest average insert size (541bp) for a batch consisting of blood-derived DNA samples within the recommended input range. The remaining sites showed average insert sizes within the 469-528 bp range, with a small number of outliers observed in buccal or saliva-derived DNA samples (Site #1 and Site #5). Across all sites, insert size met the predefined acceptance criterion of ≥ 400bp insert size, supporting optimal sequencing efficiency on Illumina platforms (Figure 8).

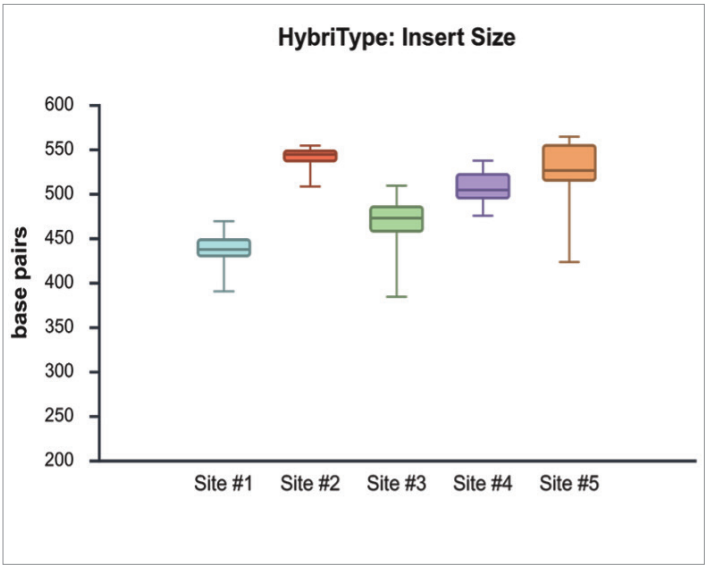



**Figure 8.** Average insert size for DNA samples after adapter trimming ranged from 439 to 541bp across all test sites.

## Duplication Rate

Duplication rate refers to the percentage of sequencing reads that originate from the same DNA fragment and are therefore considered redundant. Duplicate reads typically arise during library amplification (PCR) or from over-sequencing of a limited pool of unique fragments. In bioinformatic analysis, duplicate reads are identified based on identical alignment positions and orientation. High duplication rates indicate limited fragment diversity (low library complexity) and potential over-amplification, whereas low duplication rates reflect high fragment diversity (high library complexity) and efficient library preparation. Additional factors that may contribute to increased duplication rates include suboptimal DNA input quantity and reduced DNA quality. Across all testing sites, average duplication rates ranged from 3% to 24% (Figure 9). Sites with smaller sequencing batches (n=24 samples) displayed low duplication rates of 3% and 5%, whereas testing sites processing larger batch sizes showed duplication rates of 24%, 10%, and 11%. Site #1 showed the highest duplication rate (24%), likely attributable to lower DNA input amounts and reduced sample quality. Furthermore, DNA input quantities at Site #1 may have been overestimated due to reliance on Nanodrop™ spectrophotometry for quantification.

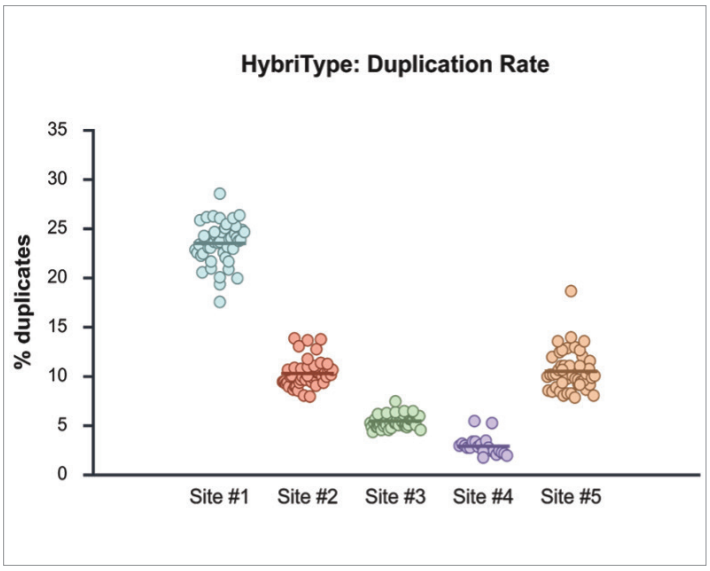



**Figure 9.** Average duplication rate for clinical samples evaluated with the HybriType Kit.

## Conclusions

As NGS-based HLA typing solutions continue to evolve, workflow complexity, data quality, and cost-effectiveness remain key factors influencing laboratory adoption. The HybriType RUO Kit helps provide a hybrid capture-based target enrichment alternative that combines streamlined workflow design, flexible and comprehensive gene content, copy number variation (CNV) assessment, and compatibility across four Illumina sequencing platforms.

This muti-center evaluation across five independent HLA typing centers demonstrates that the HybriType RUO Kit helps deliver reliable HLA typing results with minimal ambiguity, strong sensitivity across targeted regions, high specificity characterized by robust on-target enrichment, and high library complexity evidenced by low duplication rates.

Comparable to the performance of comparator devices used by participating centers, the HybriType Kit enabled expanded genotyping of non-classical HLA loci, MICA, MICB, and ABO- targets not routinely assessed but potentially valuable for supporting understanding transplant research outcomes and immunogenetic insight.

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