Quality Metrics evaluation for 05 NGS HLA typing kits

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INTRODUCTION

Quantitative and qualitative improvements of Sanger to massive parallel sequencing transition have motivated HLA typing services to look for the best HLA typing protocol available.

Sanger-NGS agreement analysis helps to **determine accuracy rate**;

Quality metrics profile evaluation will **determine result reliability**.

High polymorphism of classical HLA genes demands not only a **secure margin between maximum sequencing noise level and heterozygosity percentage** threshold but also a **well-balanced allele representation** on heterozygous, avoiding HLA typing mistakes.

OBJECTIVE

To determine quality metrics profile of five commercial NGS HLA typing kits.

METHODS

HLA-A, -B, -C, -DRB1, -DQB1, -DPB1 sequencing data from **1330 DNA samples** were generated **using five NGS HLA typing commercial kits** by two different HLA typing services as described on tables 01 and 02. Reads were aligned to reference sequence (v. 3.30.0; IMGT) using a commercial software (NGSEngine, GenDx).

Quaity metrics Percentage level of maximum noise (MNL), delta signal to noise (DSN) and estimated second allele (ESA) were calculated for exonic region. ESA less than 30% were considered unbalancing occurrences.

NGS platform	Protocol	INCA Samples	INCA Results	Pio XII Samples	Pio XII Results
Ion S5	NxType	32	192	93	558
	AllType	96	750	0	0
MiSeq	HoloType	0	0	56	327
	Trusight	0	0	186	2735
	NGSGo	0	0	867	1104

Table 01: Samples and result count separated by center and protocol

HLA gene	HLA typing commercial protocol: Alltype®	NxType®	HoloType®	NGSGo®	Trusight®	n
A	96	125	56	453	188	918
B	96	125	56	504	190	971
C	96	125	56	469	191	937
DRB1	96	125	56	637	180	1094
DQB1	94	125	51	472	178	920
DPB1	95	125	53	200	177	650
n	573	750	327	2735	1104	5490

Table 02: HLA typing results analyzed for each kit and HLA gene.

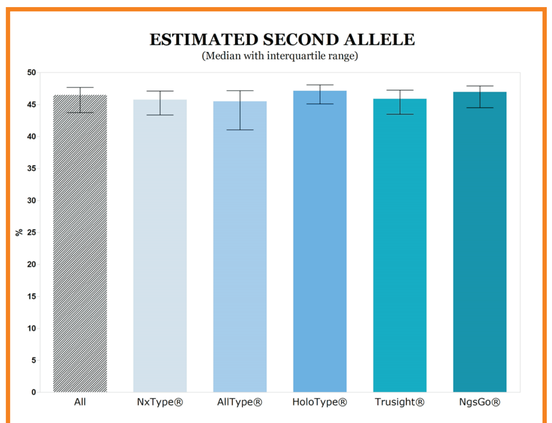
RESULTS

Kits performance analysis demonstrated **better quality metrics profile associated to Holotype and NGSgo** typings with **lower MNL** and higher ESA and DSN values when compared to NxType and AllType typings.

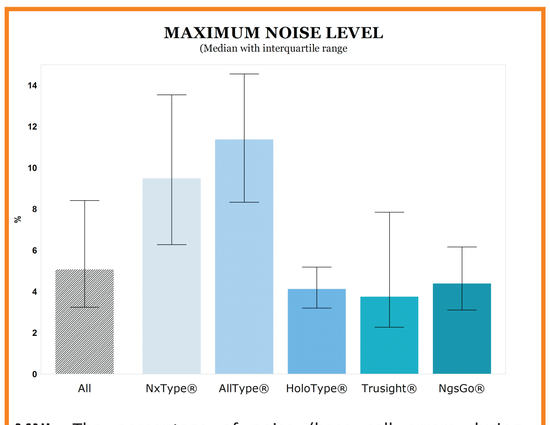
However, the **lowest allele unbalancing ratio** was detected on **NxType** (1.07%) followed by **Holotype** (2.45%) and **the highest unbalancing ratio** was found on **AllType** results (7.85%).

HLA Class I reads showed **better quality metrics** profile than Class II with **higher values for DSN** and **ESA** and **lower allele unbalance occurrences** (Class I: 5/2826, 0.53%; Class II: 201/2664, 19.57%).

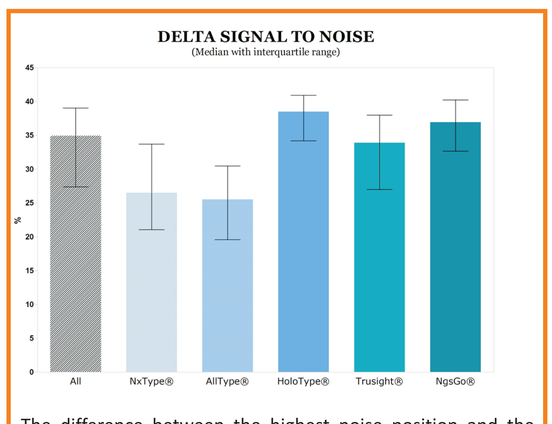
Analyzing allele unbalance distribution across HLA genes, only 3.7% results presented ESA<30% (206 in 5490 results). However, a **high ratio unbalanced x total results was found for HLA DRB1** (150/1049, 13.7%), specially **DRB1*04 alleles** (118 occurrences), and DQB1 (44/920, 4.7%)



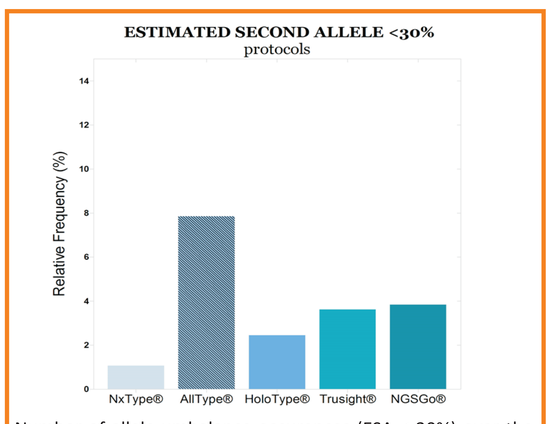
ESA: The median percentage of the heterozygous positions of the allele with the lowest abundance.



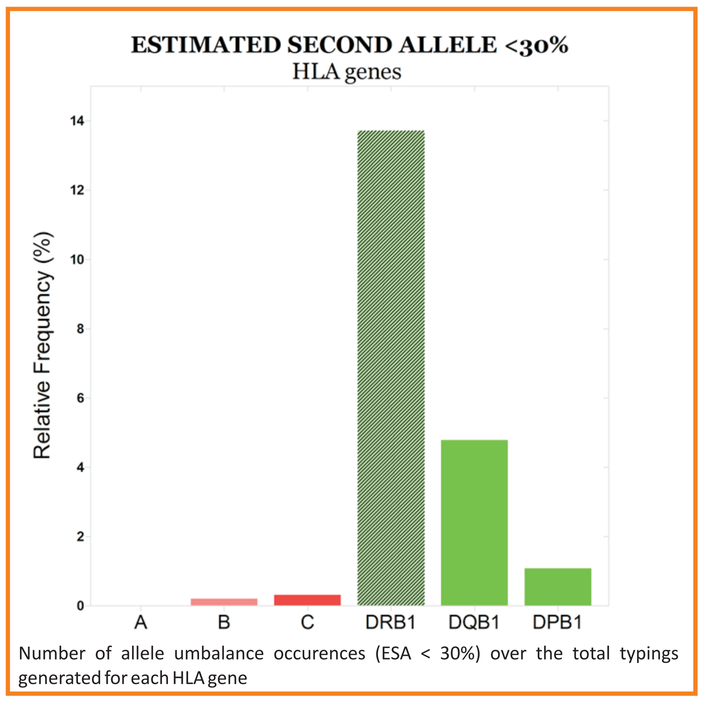
MNL: The percentage of noise (base call errors during sequencing) at the position with the highest noise level.



The difference between the highest noise position and the lowest heterozygous position in %.



Number of allele umbalance occurences (ESA < 30%) over the total typings generated by each protocol.



Number of allele umbalance occurences (ESA < 30%) over the total typings generated for each HLA gene

DISCUSSION

Differences on noise level between HLA typing Kits can be associated to NGS platform employed as literature shows that **semiconductor-based sequencing presents higher error rate than cyclic reversible termination strategy**.

However, **high allele imbalance incidence** may be **associated to amplification issues** related to primer design or protocol amplification strategy (single/multiplex).

HLA-DRB1 and -DQB1 poorer quality metrics reveals that special attention is necessary when typing those genes.

CONCLUSION

Implementation of diagnostic innovations requires a deep knowledge of its accuracy and limitations. As commercial NGS HLA kits show equally high concordance to Sanger typing, **quality metrics profile analysis can be used to figure out weak points helping to choose the best available solution.**

Projeto Gráfico: Área de Edição e Produção de Materiais Técnico-Científicos / INCA

MINISTÉRIO DA SAÚDE