DNA EXTRACTION METHOD COMPARISON FOR HLA TYPING BY NGS

THE EXPERIENCE OF NATIONAL HLA LABORATORY - BUCHAREST, ROMANIA

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- **National Institut of Hematology and Transfusion**
- **Romanian National Bone Marow Donors Registry**

National HLA Laboratory – Bucharest, ROMANIA

- Clinical categories:
  - Organ transplantation
  - Bone marow transplantation
  - Diseases associations
  - Transfusion
- Techniques:
  - PCR SSP/SSO/SBT/NGS – 2 digits/4 digits
  - Luminex - Abs screening/ identification
  - CDC - Cross-match

- Volume of annual testing
  - 1.000 patients and donors
  - 4.000 new donors for Romanian Registry
- Registry enrollment rate: 10.000/year
  - 2018 - more than 15.000 samples stored

    (75% frozen at -40˚C / 25% stored at +4˚C)

Request for a new high-throughput method

- 2018 – Tender for HLA Typing by NGS technique
- Omixon – complete solution
- MiniSeq platform - 300-cycle chemistry - fully compatible with Holotype HLA
- The tender requirement: DNA extraction to be provided → InnuPure C16 (Analytic-Yena)

Validation of NGS in National HLA Laboratory

- Period: Oct-Nov 2018

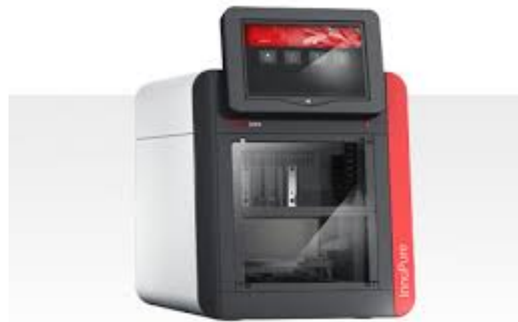
- 216 DNA samples:
  - fresh
  - frozen for 6 – 36 months at -40˚C
  - stored up to 6 months at +4˚C

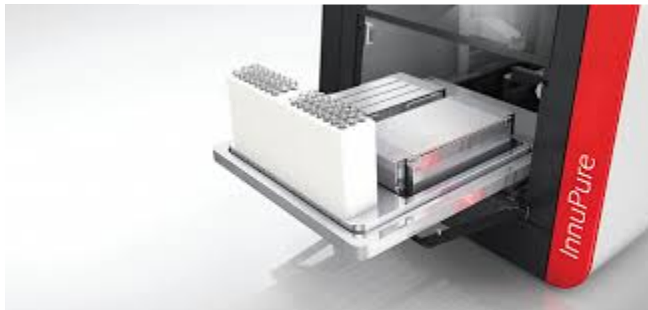
- DNA extraction: 3 machines with bead-based automated magnetic DNA extraction

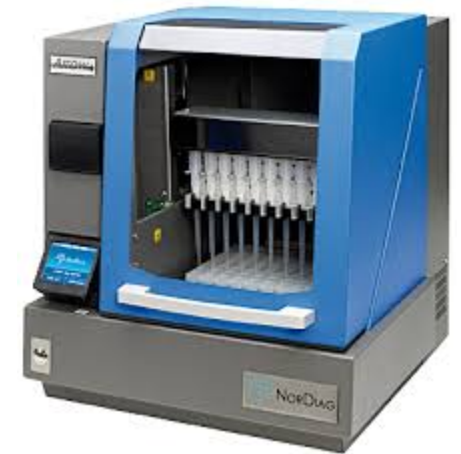
DNA extraction robots

Equipment	InnuPure	iPrep	Arrow-Nordiag
Number of samples/run	1-16	1-13	1-12
Time	45 min	39 min	45 min
Elution	**In strips(*)** / variable volume	In tubes 1,8 ml / variable volume	In tubes 1,8 ml / variable volume
DNA concentration	**9-76ng/µl**	**13-123ng/µl**	**10-53ng/µl**

(*)Lower risk of error in DNA distribution before amplification







Conclusion from the initial NGS validation for the selection of the best DNA extraction technique

Results - Poster session - P 177

- The failures : - failed of amplification due to the poor quality of some old samples
  - human errors in pooling step
- No significant correlation of the final results and DNA extraction method on the 216 samples

- Applied strategy:
  - InnuPure → Registry donors
  - iPrep → Clinical samples
    → Registry donors – only low quality samples

- Next step: Evaluation of HLA typing by NGS at three months

HLA typing by NGS – evaluation at 3 months

Material and methods:

- 1115 EDTA - Registry donors samples stored
  - Collection date range: Dec. 2017-Mai 2018
- 133 EDTA - current patients/donors samples
- Sample storage condition: +4˚C
- Testing date: Dec 2018-Mar 2019
- Runs performed by 2 technicians : 6x48 / 10x96
- DNA extraction:

  Stored samples – 784 on InnuPure

  – 337 on iPrep

  Current samples – 133 on iPrep
- DNA evaluation: Implen NanoPhotometer

- Loci tested: HLA-A,-B,-C,-DRB1,-DQB1,-DPB1(25%)
- PCR reactions and library preparation incubations - Biometra TAdvanced thermocyclers (Analytik Jena)
- The Holotype HLA v2 protocol - no deviation
- The library size selection - PippinPrep equipment
- The final library concentration - estimated on Qubit
- Mid-output MiniSeq cartridge

HLA typing by NGS – evaluation at 3 months

- DNA quality parameters

Equipment		InnuPure Stored samples N=784	iPrep Stored samples N=331 (*)	iPrep Current samples N=133 (*)
DNA Concentration	10-20 ng/µl	**144 (18%)**	13 (4%)	4 (3%)
	20-30 ng/µl	**180 (24%)**	45 (14%)	12 (9%)
	≥30 ng/µl	460 (58%)	**274 (82%)**	**117 (88%)**
Ratio	260/280	1,74-1,92	1,66-2,4	1,78-2,1
	260/230	1,2-3,84	0,98-2,8	1,72-1,98

(*)More samples needed dilution before amplification

HLA typing by NGS – evaluation at 3 months

- <u>Analysis of the amplification failures in correlation with the DNA extraction</u>

  - Input of DNA concentration for the Long-Range PCR 10 - 30 ng/µl

DNA extraction	DNA Concentration / Amplification failure	All loci	A	B	C	DRB1	DQB1
InnuPure - Stored samples	10-20 ng/µl N=144	**3,4%** N=5	**1,3%** N=2	**0,7%** N=1	0	**2%** N=3	**26%** N=39
	20-30 ng/µl N=180	**1,1%** N=2	0	0	0	**2,7%** N=5	**7,7%** N=14
	≥30 ng/µl N=461	**1,3%** N=6	0	0	0	**1,5%** N=7	**4,5%** N=21
iPrep - Stored samples	10-20 ng/µl N=13	**7%** N=1	0	0	0	0	**7%** N=1
	20-30 ng/µl N=45	**2,2%** N=1	0	0	0	0	**6,6%** N=3
	≥30 ng/µl N=274	0	0	0	0	**1,8%** N=5	**1,0%** N=3
iPrep -Current samples	10-20 ng/µl N=4	0	0	0	0	0	50% N=2
	20-30 ng/µl N=12	0	0	0	0	0	0
	≥30 ng/µl N=117	0,8% N=1	0,8% N=1	0,8% N=1	0,8% N=1	0,8% N=1	0

Discussion

- There are many parameters / steps influencing the results on each testing method, also applicable to the NGS technique – the quality of DNA is only one of them, which primarily affects the amplification.
- InnuPure machine can extract a good quality DNA immediately available in 8-well strips for easier manipulation.
- iPrep machine can provide a superior quality of DNA with higher yield reaching less PCR failures.
- In our experience, the amplification for HLA Class I loci was good, even for samples with low DNA concentration, with no differences between the two extraction methods used.
- The low concentration of DNA affected mostly HLA class II amplification, the DRB1 and especially the DQB1 locus, despite the good quality and good 260/280 ratio of most of them.
- Regarding the failed amplifications of DNA with concentration ≥30 ng/µl, these samples were among those with the lowest 260/280 and 260/230 ratios, this is the limit of the extraction methods available in our laboratory.

Conclusion

- Regarding to our expectations of the extraction method – robustness and quality - we chose to combine two DNA extraction methods in routine:

- InnuPure to be used for large number of samples - even if it has a lower DNA yield, we took into consideration the advantage of elution into the strips, that reduces the risk of handling errors.

- iPrep, with superior extraction yield, is more suitable to be used for daily blood samples, especially for leukemic patients, patients with aplasia, and also for old blood samples with low DNA quality.

Thank you !