

A Day in the Life of a Transplant Center Lab

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Disclosures

The following faculty and planning committee staff have the following financial disclosures:

Name	Institution
Neng Yu, MD	American Red Cross UMass Memorial Medical Center
Miranda Bauer, PhD	National Marrow Donor Program
Bernadette Anton R.N. BSN	National Marrow Donor Program



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Two Learning Objectives

- Analyze what **comprehensive** HLA support the stem cell transplant program can expect to receive from a testing laboratory
- Evaluate how a **close** HLA and HCT relationship can **enhance** the program performance

What Is Your Current TC-HLA Dialogue?

Sound familiar?

1. It's been a week! Where is my result?
2. Did you receive the sample?
3. Can you run this STAT?
4. What does the result mean?
5. Did I order the right test?
6. Did I activate the right donors?
7. Our marrow clinic is Friday, can you run CD34 chimerism on Saturday?

What's Possible

How about requests like this?

- Patient condition changed; I am sending typing today. Can I have the results tomorrow?
- Difficult donor search; can you try?
- Running desensitization; STAT antibody on Tuesday and Friday ok?
- Last year, testing cost was too high; are there potential savings without lowering quality?
- Epic-HistoTrac interface in place in 2018?

Accessibility



- Sample receiving 24/7/365
- 6-day operation: Monday through Friday 6AM - 8PM, Sat 7AM- 3:30PM
- Comprehensive HLA test menu: Pre-, Peri-, and Post-HCT tests
- Director consultation: donor selection, data interpretation, support recommendation, etc.

Menu



HLA Typing:

Gene: HLA-A, -B, -C, -DRB1, -DRB345, -DQA1, -DQB1, -DPA1, -DPB1; individually or in any combination as needed.
Resolution: low-intermediate, high, allelic.
Method: NGS, Sanger, PCR-SSP, and PCR-SSOP

HLA Antibody Analysis:

Specificity identification: Class I and II.
Method: Luminex, single antigen beads, C1q, DSA and titer.

Menu



T and B Cell Crossmatch:

Method: CDC, AHG-CDC.

Disease Association/Drug Sensitivity:

Gene: any HLA genes of interest associated with any disease or drugs.

Method: low-resolution reflex to allele if positive

Engraftment Monitoring:

Cell type: whole blood, T cells, B cells, Myeloid cells, myeloid progenitor cells, NK cells, whole marrow, CD34 (Progenitor marrow cells).

Method: PCR-STR, 24 markers

Menu



Platelet crossmatch:

Solid phase red cell adherence assay.

Platelet Typing:

Gene: HPA-1 (PLA-1), low resolution by PCR-SSP.

KIR Genotyping:

Gene: 2DL1-5, 2DS1-5, 2DP1, 3DL1-3, 3DS1, 3DP1.

Genotyping, allele level typing upon request.

Method: PCR-SSOP, PCR-SSP, SBT.

Miscellaneous:

Histocompatibility-related research tests, such as CCR5-Δ32 screening, DPB1 rs9277534 Expression Marker, etc.

Test Selection-When and What?

- Recipient:
Transplant Work-up-All loci allelic typing by NGS **when allo HCT option chosen**; HLA antibody analysis by Single Antigen Bead (SAB)
- Related Donor: HLA-A/B/DRB1 intermediate resolution, reflex to Transplant Work-up if matched or haplo matched
- Unrelated Donor: Transplant Work-up
- Cords: Transplant Work-up

Test Selection-When and What?

- CT to confirm identity

HLA-A/B/DRB1 intermediate resolution- Recipient/RD prior to final donor selection

- CT to confirm homozygosity

Active cancer patient, blood specimen is discouraged for genetic analysis. If used in Transplant Work-up and being homozygous, CT by Transplant Work-up using swab.

- Antibody analysis frequency

At the beginning of donor selection, 3 weeks post sensitization event, within 30 days of transplant, and every 6 months for lengthy search process.

- KIR?

If multiple donors found, if 2nd HCT due to relapse, if AML/MDS, if...

Turn Around Time (TAT)

Schedule you needs, Systematic process, Timely communication

- Bone Marrow clinic date/time- **Schedule**
- Weekly QAPI date/time- **Schedule**
- **Systematic Process** not 'a la carte'
- Group email, not individual contact, **reach everyone or someone and leave no room for lacking timely communication...**

Turn Around Time (TAT)

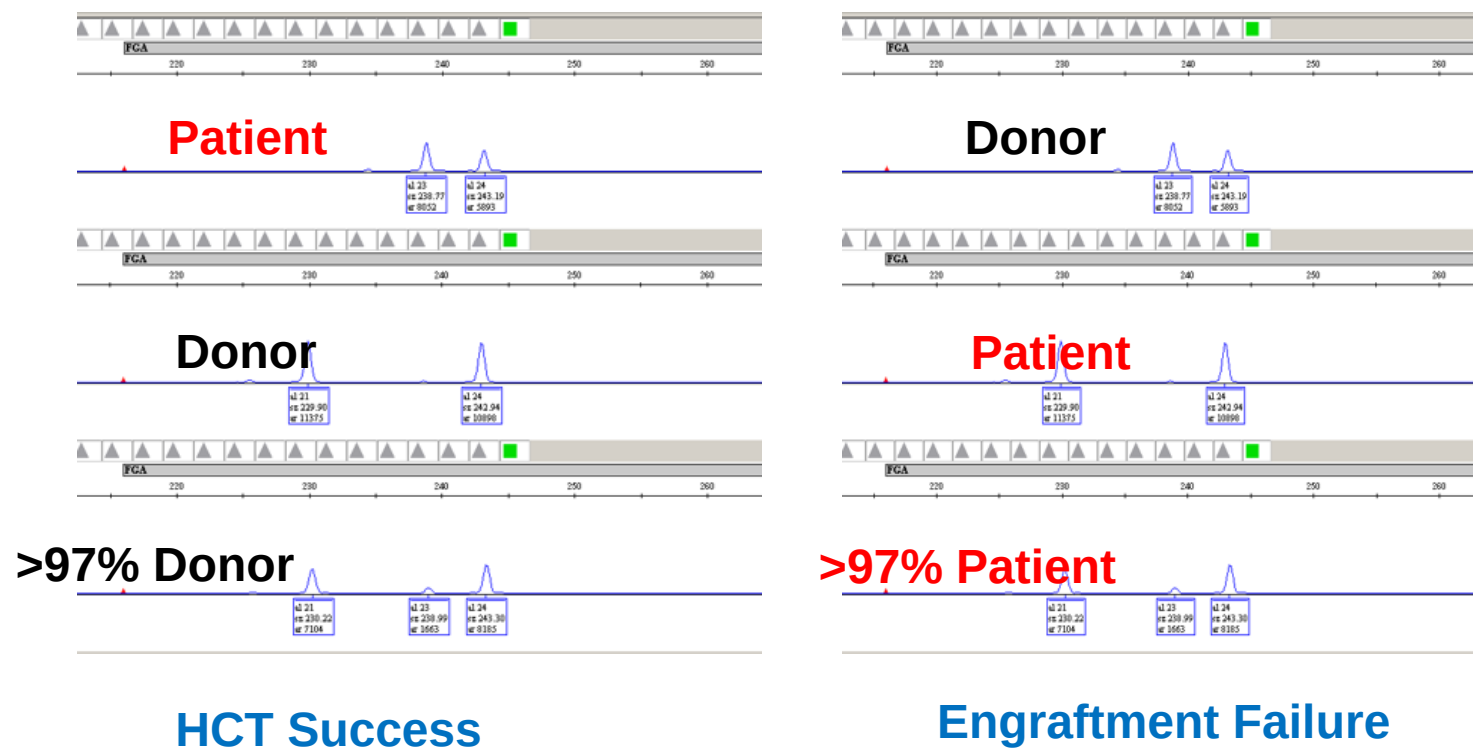


- Same day: Antibody, crossmatch
- Two day: Chimerism
- Three day: HLA typing

All samples received before 11am. Adequate sample volume for daily setup

- **Day 1:** FedEx-10:30 am, log in/Batch extraction/SSOP and NGS PCR setup
- **Day 2:** SSOP analysis, NGS library prep and sequencing
- **Day 3:** NGS analysis, SSO/NGS data sync, HistoTrac report

It's So Easy to Make a Devastating Mistake!!



Effective QA/QC

DO MORE THAN REQUIRED



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How to QA/QC Chimerism Analysis:

1. Two Known % controls for quantitative accuracy.
2. Identity control: pre-HCT patient and donor DNA are run with post-HCT samples every time.
3. Patient's STR profile is confirmed twice using the two samples mandated for HLA typing.

Effective QA/QC

DO LESS...



A new lot of Luminex SSO kit arrived...

1. Is the kit used as stand alone method? Solid organ, disease association, etc.
2. Are any samples on the run parallel tested by a 2nd sequencing method?
3. What has changed with the lot? Primer? Probes? Database?

Less QC performed if:

- a. >90% of samples are tested by a 2nd method, parallel can be used as QC.
- b. Parallel data also serve for database validation, primer change validation for coverage, probe performance validation since parallel methods are at higher resolution.
- c. No results are released until parallel data examined, concordance or root cause analysis performed if discordant.

Do the right thing

- **Common Sense**
- **Science! Understand why the rule instead of memorize the rule**
- **How to handle FUD moment (fear, uncertainty and doubt)? STOP and Trace back**
- **Communicate, and communicate TIMELY**
- **Fault the carelessness and recklessness. Do not fault the incorrect conclusion of a carefully analyzed problem**

A Typical Day (5:30AM-7:30PM)



Sample Management:

- Sample transportation: FedEx/DHL, ARC courier, USPS, STAT courier- **24/7/365**
- Sample processing: LIS-HistoTrac- **3PM**
- Cell subset isolation/DNA extraction: **5PM**
- PCR: **Overnight**

A Typical Day (5:30AM-7:30PM)



Transfusion Related:

- HLA matched platelet: local and national inventory search- **Before noon**
- HLA antigen avoid platelet- **Before noon**
- HLA antigen positive platelet: platelet assist desensitization 'sponge'- **Before noon**
- XM negative platelet- **Before noon**

A Typical Day (5:30AM-7:30PM)



HLA Antibody Analysis:

- Serum prep-DTT, adsorption, dilution, etc.-

Before noon

- Single Antigen Bead (SAB) assay- **PM**
- C1q binding assay- **PM**
- Antibody titer assay- **PM**
- DSA monitoring in parallel with desensitization- **PM, STAT**

A Typical Day (5:30AM-7:30PM)



HLA Typing:

- PCR-SSOP PCR- 4-6PM
- PCR-SSOP detection- LabExpress Robot overnight
- NGS PCR- Overnight
- Sanger SBT PCR (as needed)- Overnight

A Typical Day (5:30AM-7:30PM)



Chimerism Analysis:

- Cell subset isolation- on robot 1-4PM
CD3/CD4/CD8/CD19-20/CD33/CD34/CD56/CD71
- DNA extraction-blood, marrow, subsets 4-5PM
- PCR Overnight

Miscellaneous test: As needed

- KIR
- CCR5 delta 32
- HPA-1

A Typical HCT HLA Work-up



- TC: AML pt., 71Y/F, A+, CMV-, 90kg, suggestions?
- Lab: swab/HLA, sera/HLA antibody, no sib if >70Y, type kids/grandkids, search MUD.

Three days later...

- Lab: Pt highly sensitized, DSA to all Bw4s, Haplo risky, here are the MUD choice:
 1. 1111-1111-1 (F22 Opos, CMV neg), 73kg
 2. 2222-2222-2 (M25, Opos, CMV neg), 93kg
 3. 3333-3333-3 (M24, Opos, CMV neg, 83kg

All 10/10, antibody N/A, she is AML, KIR as the tie-breaker?

Example of Haplo Donor Selection Checklist

Discuss with HCT team, then share the general guideline with the lab, e.g.,

- Age: <32, <38, <40, and risk increment by each year
- Avoidance of offspring donors for maternal recipient
- Avoidance of female donors for male recipient
- KIR 2DS2 may be beneficial in myeloid malignancies to ↓NRM and ↑OS.
- KIR L-MM
- >4MM (10/10 setting) in GvH to ↓Relapse
- CMV+ recipient with CMV+ donor

Example of Donor Option Preference

1. HLA matched sibling (<50Y)
2. HLA 10/10 matched MUD
3. Haplo>Cord=9/10 MUD with DQB1
MM>9/10 MUD with non-DQB1 MM



HLA Services
180 Rustcraft Road
Suite 115
Dedham, MA 02026
(tel) 781-451-2148
(fax) 781-451-2269

Blood Services
East Division
ASH# 10-1-MA-01-1
Director: Neng Yu, M.D.
CLIA# 22D0073830
Director: Jorge Rios, M.D.

Stem Cell Recipient

HLA Report

Patient: 
DOB: 
MRN: 
Category: Stem Cell Recipient
Report Date: 10/13/2017

Report to: 

HLA Typing

Name

DOB/MRN

LID

Relation/ Source

Sample Dt
Receive Dt
Test Dt

A*

B*

C*

DRB1*

DRB345*

DQA1*

DQB1*

DPA1*

DPB1*

Haplo

07/13/2017
07/14/2017
07/17/2017

02:01

37:01

06:02

10:01

01:8XYG

05:01

02:01

03:01

40:01

03:04

13:02

3*03:01

01:AR3W

06:04

04:01

07/25/2017
07/26/2017
07/28/2017

02:01

37:01

06:02

10:01

01:8XYG

05:01

03:01

03:01

40:01

03:04

13:02

3*03:01

01:AR3W

06:04

07/31/2017
08/01/2017
08/03/2017

02:01

37:01

06:02

10:01

01:8XYG

05:01

02:01

03:01

40:01

03:04

13:02

3*03:01

01:AR3W

06:04

04:01

08/01/2017
08/02/2017
08/04/2017

02:01

37:01

06:02

10:01

01:8XYG

05:01

02:01

03:01

40:01

03:04

13:02

3*03:01

01:AR3W

06:04

04:01

08/03/2017
08/07/2017
08/09/2017

02:01

37:01

06:02

10:01

01:8XYG

05:01

02:01

03:01

40:01

03:04

13:02

3*03:01

01:AR3W

06:04

04:01

Comments:

HLA match is evaluated based on HLA-A/B/C/DRB1/DQB1 10 alleles.

HLA-DPB1 TCE Algorithm v2.0 (2016-08): Biol Blood Marrow Transplant (2015) 21:233-41

Confirmatory typing (CT) is performed for HLA-A, -B and -DRB1.

MM=mismatch; DSA=patient carries Donor Specific Antibody.

ARS=Antigen Recognition Site, mismatch outside ARS carries minimum immunological risk.

- 07/17/2017: Recipient work-up-swab 07/13/2017.
- 07/28/2017: 1111-1111-1: 10/10, DPB1 non-permissive HvG MM.
- 08/03/2017: 2222-2222-2: 10/10, DPB1 match.
- 08/04/2017: 3333-3333-3: 10/10, DPB1 match.
- 08/09/2017: 4444-4444-4: 10/10, DPB1 match.
- 09/12/2017: 5555-5555-5: 10/10, DPB1 permissive MM.
- 10/10/2017: Recipient CT-blood 10/08/2017, typing confirmed.

KIR

⊕ **KIR genotyping** (performed by PCR-SSP method)

KIR Ligands* / KIR genes 1 = KIR gene present, 0 = KIR gene absent		C2	C1	C1	HLA-G		Bw4	HLA-A1, A11			C2							Donor KIR B-content
		2DL1	2DL2	2DL3	2DL4	2DL5	3DL1	3DL2	3DL3	3DS1	2DS1	2DS2	2DS3	2DS4	2DS5	2DP1	3DP1	
RDonor: 2222-2222-2 Collection date: 07/31/2017 Received date: 08/01/2017	Test date: 10/11/2017	1	1	1	1	0	1	1	1	0	0	1	0	1	0	1	1	NEUTRAL
Donor: 3333-3333-3 Collection date: 08/03/2017 Received date: 08/09/2017	Test date: 10/11/2017	1	1	1	1	0	1	1	1	0	0	1	0	1	0	1	1	NEUTRAL
Donor: 4444-4444-4 Collection date: 08/03/2017 Received date: 08/09/2017	Test date: 10/11/2017	1	1	0	1	1	1	1	1	0	0	1	1	1	1	1	1	BEST

* KIR C1 Ligand = HLA-C alleles with Asparagine (Asn-AAC) at codon 80
KIR C2 Ligand = HLA-C alleles with Lysine (Lys-AAA) at codon 80 (<http://www.ebi.ac.uk/ipd/kir/ligand.html>)

HLA /KIR Ligands

Recipient		A*		B*		C*	
Patient	Typing	02:01	03:01	37:01	40:01	03:04	06:02
	Ligand		A3	Bw4		C1	C2

10/12/2017: The patient is AML with HLA-C1/C2 KIR ligand profile, may have higher risk of relapse than HLA-C1/C1 patients.

Donor, 2222-2222-2 KIR profile:

- 2DS1and 3DS1 Negative
- There is no inhibitory KIR missing ligands in recipient
- Neutral KIR B-content

Donor, 3333-3333-3 KIR profile:

- 2DS1and 3DS1 Negative
- There is no inhibitory KIR missing ligands in recipient
- Neutral KIR B-content

Donor, 4444-4444-4 KIR profile:

- 2DS1and 3DS1 Negative
- There is no inhibitory KIR missing ligands in recipient
- BEST KIR B-content

Donor 4444-4444-4 has the best NK-KIR allo reactivity.

HLA Report

HLA Antibody Analysis

cPRA:

<u>Sample Dt / Receive Dt / LID</u>	<u>Test Dt</u>	<u>Screen</u> <u>Class I</u>	<u>Screen</u> <u>Class II</u>	<u>SAB*</u> <u>Class I</u> <u>(MFI>4,000)</u>	<u>SAB*</u> <u>Class II</u> <u>(MFI>4,000)</u>	<u>C1q</u> <u>Class I</u>	<u>C1q</u> <u>Class II</u>	<u>Dilution</u> <u>Class I</u>	<u>Dilution</u> <u>Class II</u>	<u>Comments</u>
10/09/17 / 10/10/17 / R606258-7	10/10/2017			A:32 B:7 8 13 18 38 39 41 42 48 49 51 52 54 55 56 57 58 59 60 61 63 67 78 81 82 Cw:15		B:7 8 13 18 38 39 41 42 48 51 52 54 55 56 57 59 60 61 63 67 78 81 82 Cw:15				
10/02/17 / 10/03/17 / R606258-6	10/10/2017			B:7 8 13 18 38 39 41 42 48 51 52 54 55 56 57 58 59 60 61 63 67 78 81 82 Cw:15						

Grey Zone Antibodies-GZA (MFI 1,500-4,000): A:23 24 25
B:35 37 45 53 64 65 71 73 77 2708

Unacceptable antigen is listed based on individual tested serum. Grey Zone antibodies are cumulative.
HLA antibody analysis was performed by Luminex LABScreen Mixed , LABScreen PRA, LABScreen Single Antigen (SAB).

Donor Specific Antibody (DSA) Monitoring

<u>Sample Dt / ID</u>	<u>Donor Name</u>	<u>Test</u>	<u>DSA (MFI)</u>	<u>Comments</u>
10/09/2017 / R606258-7		A-LumSAB1	A24(600), B39(16,400)	
10/09/2017 / R606258-7		A-LumC1Q SAB1	B39(14,500)	
10/02/2017 / R606258-6		A-LumSAB1	A24(700), B39(15,000)	

Comments

A Close TC-HLA Relationship

- Ask what you need, don't worry if possible
- Both sides receptive for feedback
- Practice review, annually at minimum
- Request/receive lab presentation, CE option for residents/nurse/staff
- Joint report review and tailor donor selection with clinical condition and treatment plan

Centralized Histocompatibility Testing

- ARC Dedham_HLA is also a **Centralized Histocompatibility Testing lab** for patients, related donors, and unrelated donors
- **Key Services:**
 - 3 day laboratory turnaround time
 - Allele level HLA testing
 - HLA Antibody analysis, chimerism, and crossmatching available
 - Automatic reporting of form 22's and 117's

Centralized Histocompatibility Testing

HLA testing and other non-HLA factors

- Allele level HLA-A,-B,-C,-DRB1,-DRB3/4/5,-DQA1,-DQB1,-DPA1,-DPB1
- Intermediate resolution HLA-A, -B, -DRB1, etc.

Other testing options

- KIR and CCR5 Δ 32 mutation
- Antibody screening including PRA, Single Antigen Bead, titration and C1q
- Crossmatch including T cell and B cell
- Chimerism with numerous cell subsets



The difference between something good and something great is attention to detail.
[Charles R. Swindoll](#)

Thank You!



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