

Dear ASHI Director Candidate:

A candidate for Director of a laboratory accredited by the American Society for Histocompatibility & Immunogenetics (ASHI) must demonstrate a level of training and experience commensurate with the roles of a Director, Technical Supervisor, and Clinical Consultant. There is a requirement for a candidate to have, and communicate, a high level of understanding of the technical aspects of clinical histocompatibility testing. The DTRC must be able to state that a candidate has a clear understanding of the testing being done, how the results are interpreted, the limitations of the testing, and the possible clinical implications.

The portfolio of cases is an opportunity for a candidate to present interesting cases in which the candidate was the primary reviewer. Each case presentation should contain a detailed summary and supporting worksheets reviewed by the candidate. The case summary should have a case identifier that links the summary to the worksheets and include:

- The accreditation category (e.g. Solid-organ related, HSC/BM unrelated, Disease-association, etc.).
- Relevant patient demographics and clinical history.
- The technologies and tests used to evaluate the case.
- The results from the testing.
- The candidate's interpretation of the results.
- Possible problems, concerns, or limitations.
- Clinical outcome, if known.

The worksheets should contain the initials and date of review by the candidate.

In a histocompatibility laboratory, the interpretation and application of clinical testing often depends on the procedures and policies in place at the time of testing. Copies of the major procedures and/or policies, written or revised by the candidate, will assist the reviewer(s) in evaluating a candidate's conclusions; these can be submitted with the portfolio.

A candidate seeking approval for an area of technology used in the laboratory (e.g. flow cytometry, serology/solid phase, SBT, etc.) must submit documentation of expertise for each area of technology. Documentation can include:

- Validation package accepted by ASHI.
- Participation by the candidate in external proficiency testing.
- Procedures written by the candidate.
- Evidence of training others to do the testing.
- Evidence of quality control activity.

Please see the following examples as a template for detailed case studies.

**CASE PORTFOLIO FILE FOR HISTOCOMPATIBILITY (HLA)
LABORATORY DIRECTOR TRAINING AND QUALIFICATION**

Case Number:

4.5 patient RJ869

Category or Type of Case:

4 Solid Organ-Live Donor

Blood Group:

Recipient: B Rh positive

Donor: O Rh positive

Ethnicity: Asian, native of Philippine

Gender: Female

Organ type: Kidney

Technology(ies)/Test(s) Used:

- HLA typing SSP
- HLA typing r-SSO
- Flow cytometry T and B cell crossmatches
- T-cell CDC-AHG crossmatch
- Single antigen Bead (SAB) testing for antibodies to HLA Class I and II

Diagnosis:

End-stage renal disease secondary to AL amyloidosis status post renal transplant in 1988 and status post autologous stem cell transplant in 2006 and 2008.

Clinical History:

This is a 51-year-old female with a history of AL amyloidosis that developed after living related donor kidney transplantation. She received a living donor kidney transplant from her brother in 1988 in Saudi Arabia for end-stage renal disease of unclear etiology. She did well with this kidney until March, 2005 when she developed allograft pyelonephritis. After treatment with antibiotics, her renal function failed to recover, and a renal biopsy

showed AL amyloidosis with lambda light chain staining. She underwent an autologous stem cell transplant on March 3, 2006. Despite what appears to be a complete hematologic response, her renal allograft function started to decline. Repeat biopsy showed only amyloidosis without any evidence of rejection. Kidney transplant evaluation was initiated. She then went on to dialysis in February, 2008. She received her second peripheral stem cell transplant in August, 2008 and appears to be in remission. Her medical history is also significant for chronic hepatitis B and C. These two infections appear to have occurred following her first kidney transplant. Due to a positive crossmatch with her potential donor, her daughter, she was evaluated to undergo a paired exchange transplant. She received her transplant on 8/14/09 from donor-3. This donor is same as donor-8 from case #4.4. Her daughter (donor-2) is the same as donor-9 from case#4.4 and she donated her kidney to recipient MB916.

Sensitization History:

- Previous transplant
- Pregnancy
- Multiple blood transfusions

Tests Performed

HLA-typing low resolution by SSP
 HLA-typing low to medium resolution by r-SSO
 SAB testing on Luminex platform for antibodies to HLA Class I and II
 Flow cytometric crossmatch: T and B Cell
 T cell AHG-CDC crossmatch

Results of Evaluation and Testing:

First Kidney Transplant performed in 1988

Patient received kidney transplant from her brother in 1988. This was performed in Saudi Arabia. No records are available for review. Patient was not sure of the exact institution and thus no additional information can be obtained.

Second Transplant:

Summary of Donors Evaluated:

- Total Number of Donors Evaluated: Three
- HLA typing performed on all three donors.
- Evaluation crossmatch performed on 2/3 donors.
- Evaluation crossmatch includes: T-cell AHG-CDC, T-cell FXM and B-cell FXM
- Final and post transplant crossmatch includes: T-cell FXM and B-cell FXM
- Donor selected to provide kidney: Donor-3
- Donor-3 was part of the first paired donor exchange program within our institution
- All HLA-typing and crossmatch results are summarized on page # 1. Pertinent results are discussed next.

Donor-1HLA typing

Results are summarized below. Worksheets are page # 66-69.

Donor-1 is the niece and is a 3/6 antigen mismatch at HLA-A, B and DR.

Comments:

Donor types as HLA-B55, 75. HLA-B75 is uncommon in the Caucasian population but its frequency ranks #10 in the Asian (API) group in US population. Donor is of Asian ethnicity.

Crossmatch

Donor not available and thus not performed.

Comments:

Recipient SAB demonstrates rising anti-HLA-A24 since April, 2008. SAB from her sample dated 5-11-09 (page# 37) demonstrates antibodies to HLA-A24; highest MFI=10124. Based on our experience as explained below, one can predict a positive T-cell and B-cell FXM.

Donor-2HLA typing:

Results are summarized below. Worksheets are page # 70-73.

Donor-2 is the daughter and is a 3/6 (haplotype) match at HLA-A, B and DR.

Comments:

- The likely HLA-DR-DQ haplotypes are DR12-DR52-DQ7 and DR15-DR51-DQ5 based on the following reason:
 1. The DR12-DQ7 linkage disequilibrium is common except in African American (AFA) population. In AFA, 21% demonstrate the DR12-DQ7 linkage while 79% demonstrate DR12-DQ5 linkage.
 2. The most common linkage disequilibrium is DR15-DQ6. However, DR15-DQ5 linkage has been reported. In fact amongst the Asian population 65% demonstrate DR15-DQ6 and 35% demonstrate DR15-DQ5.
 3. Donor-9 is a native of Philippine.
- The likely HLA-Cw-B haplotypes are Cw9-B18 and Cw8-B75. Although, Cw9-B18 haplotype is uncommon in the European, Hispanic and AFA population, it is seen in the API population. Also B75 frequency ranks #10 in the API group in US population.

Crossmatches:

Evaluation crossmatch results are summarized in the table below, positive results are in red. For T-cell FXM: cutoff: 52 MCS, B-cell FXM: cutoff: 106 MCS. Column DSA has the DSA specificity and MFI within parenthesis. Worksheets for pertinent crossmatches are on page #14-26.

Date	T-cell MCS	B-cell MCS	T-AHG-CDC	DSA (MFI)
04/02/08	39	154	Not done	DQ5 (8238)
05/11/09	2	384	negative	DQ5 (14333)

Comments:

- At our institution DSA MFI >4000 correlates with a positive FXM and DSA MFI

values <300 correlate with a negative FXM. Between 300 and 4000 MFI as the value increases there is higher likelihood of a positive crossmatch.

- Thus both the above results are consistent with the SAB results.
- This patient-donor pair participated in the first paired exchange performed within the institution. The pairs initially evaluated were:
 1. Recipient RJ (this case; #4.5) and donor-2 and
 2. Recipient BM (case #4.4) and donor-8
- This recipient has DSA to donor-2 and a positive B-cell FXM (MCS=384). However, recipient has negative T-cell and B-cell FXM with donor-3 and thus donor-3 provided kidney to the recipient.
- Recipient BM (case #4.4) has two DSA to donor-3 including DR53 but only one DSA to donor-2 with similar B-cell FXM results. Thus donor-2 provided kidney to this recipient BM.
- Final patient –donor pairs transplanted were:
 1. Recipient BM (case#4.4) with donor-9 and
 2. Recipient RJ (this case) with donor-8.

Donor-3

HLA typing:

Results are summarized below. Worksheets are page #74-75.

Donor-3 is the unrelated living donor who is part of the paired exchange and is a 5/6 antigen mismatch at HLA-A, B and DR.

Crossmatches:

Evaluation and final crossmatch results are summarized in the table below, positive results are in **red**. For T-cell FXM: cutoff: 52 MCS, B-cell FXM: cutoff:106 MCS. Column DSA has the DSA specificity and MFI within parenthesis.

Worksheets for pertinent crossmatches are on page # 2-13.

Date	T-cell MCS	B-cell MCS	T-AHG-CDC	DSA (MFI)
<i>Evaluation</i>				
05/11/09	17	18	Not done	Cw6 (1095)
<i>Final</i>				
08/13/09	-3	-6	Not done	Cw6 (508)

Comments:

- As explained above, the flow cytometric crossmatch results are consistent with the SAB results.
- Additionally, HLA-Cw has variable expression on peripheral cells.
- As explained above, this patient-donor pair is a result of the first paired exchange within our institution.

Summary of Antibody Detection by SAB:

For patients on the living donor protocol, our current practice is to screen recipients with SAB at the initial evaluation and subsequently every quarter or sooner if there is a sensitizing event/clinical need on the pre-transplant (48 hours old or less) serum sample. In the living donor cases, SAB are also run on samples that are used to perform evaluation crossmatches.

Summary of Screening SAB's (page #31-65):

Sample from 4-4-08 to 07-02-09 are screening SAB and demonstrate multiple anti-HLA Class I and II antibodies; Class I: A23, 24, Cw6; Class II: DQ5, DQ6.

Comments:

SAB test could identify antibodies to HLA-Cw since May, 2009 with the implementation of Combi beads (One Lambda). HLA-Cw is the DSA and MFI from SAB after May, 2009 are listed above.

Recipient types as DQ6, however, the SAB demonstrate antibodies to DQ6. One can hypothesize that these antibodies are allele specific. This is demonstrated by the fact that in her samples from June, 2008 to April, 2009, the bead with highest MFI is the one with DQB1*0603, while her most recent samples from August and July, 2009 demonstrate that the bead with highest MFI is the one with DQB1*0609. In all samples the bead with DQB1*0601 has a 0 or low MFI. Additionally beads with DQ and DP specificity are a combination of DQA1 and DQB1 epitopes. Thus, the other possibility can be that the antibody recognizes both DQA1 and DQB1 chains and possibly the DQA chain merely modifies the DQB epitope. We do not have high resolution typing on the recipient, but based on the antibody profile, one can speculate that she is a DQB1*0601.

Summary of immediate pretransplant SAB (page# 27-30).

Sample from 8-13-09 (page#24-27) is the pre transplant sample and demonstrates multiple anti-HLA Class I and II antibodies. DSA is Cw6: MFI=508.

Comments:

SAB results are consistent with the negative flow crossmatch.

Summary of posttransplant SAB

No post transplant SAB performed.

Additional information

This was the first paired exchange within our institution.

This recipient clearly benefitted as her original donor was the daughter to whom she was alloimmunized with a positive flow crossmatch. However, she was not alloimmunized to the donor that she received as a part of the exchange. This resulted in a potential crossmatch positive transplant to become a crossmatch negative transplant.

The recipient also has chronic HBV and HCV and using her original donor would mean requiring higher immunosuppression which can further complicate her HBV and HCV infection status.

For the other recipient, the benefit was receiving an organ to which he has only one DSA as compared to his original to which he had two DSA.

Comments on Procedure/Testing:

Various techniques have been used and illustrate that Luminex SAB is most sensitive. However, SAB does not cover all the antibodies and thus additional testing like crossmatching is useful.

Interpretation/Consultation:

- Donor-2 is the daughter and results in a positive crossmatch due to high MFI DSA
- Donor-3 is the most compatible donor resulting in a negative crossmatch
- Case demonstrates that paired exchange can be performed within the institution

Follow Up

Considering that the patient also has chronic HBV and HCV infection, she was induced with Simulect and maintained on Prograf, cellcept, and steroid immunosuppression. Donor and graft are doing fine after 23 days.

Signed:

**CASE PORTFOLIO FILE FOR HISTOCOMPATIBILITY (HLA)
LABORATORY DIRECTOR TRAINING AND QUALIFICATION**

Case Number:

5.5 patient SB125

Category or Type of Case:

5 Solid Organ-Deceased Donor

Blood Group:

Recipient: B Rh positive

Donor: O Rh positive

Ethnicity: White

Gender: Male

Organ type: Liver

Technology(ies)/Test(s) Used:

- HLA typing r-SSO
- T-cell AHG CDC crossmatch
- Flow cytometry T and B cell crossmatches
- Single antigen Bead (SAB) testing for antibodies to HLA Class I and II

Diagnosis:

Recurrent Primary Sclerosing Cholangitis (PSC) in transplanted allograft

Clinical History:

This is a 50-year-old gentleman with recurrent PSC status post severe variceal bleeding and encephalopathy for orthotopic liver transplant (OLT). He received his first OLT in August, 1994 for PSC. His post transplant history was complicated by acute renal failure, cellular rejection, recurrent variceal bleeding, and portal vein stenosis requiring balloon angioplasty in 1994 and 1995. He has subsequently had bouts of recurrent PSC and was relisted in spring, 2009 due to worsening MELD score and progressive disease. On August 7, 2009, patient presented to an outside institution with severe GI bleed requiring TIPS procedure. He also had renal failure and encephalopathy. During the procedure he needed 3.34 L of

red blood cells, 3 L of FFP, six-pack platelets, and vitamin K. Patient was transferred to our institution for OLT. He received his second OLT on 8-14-09. His transplant procedure was complicated by requiring an unusually high amount of blood products including red blood cells, FFP and platelets.

Sensitization History:

Previous Transplant
Blood transfusion

Tests Performed

HLA Class I and Class II low to med resolution molecular typing by R-SSO
Single antigen bead testing on Luminex platform Class I and II
Flow cytometric crossmatch: T and B Cell
CDC crossmatches: T cell AHG

Results of Evaluation and Testing:

Summary of HLA typing (page #26-30)

Recipient received his first transplant in 1994. However, no tissue typing records are available for review.

HLA typing of the recipient and donor for current transplant is summarized on page #1.

Number of deceased donors evaluated: One.

Interpretation of typing: Donor is a 5/6 mismatch at HLA-A, B and DRB1 loci.

Comments:

As per UNOS typing (page #30) the donor is HLA-Cw*09, 10.

Low to medium resolution typing done at our institution demonstrates that the donor likely types as HLA-Cw9 at one antigen. However, at the other antigen, it is not possible to confirm Cw10 (cannot differentiate Cw*0303 from Cw*0304; page#28). As this is a solid organ transplant case, no additional testing was performed and the typing was reported as HLA-Cw*03, 03.

Summary of crossmatches performed (page #2-9)

Until June 30, 2009 for liver transplants, our practice was to perform a retrospective T-cell AHG-CDC crossmatch on the current (collected within 48 hours) pre transplant serum. Since July 1, 2009 our practice is to perform a retrospective T-cell AHG-CDC crossmatch, T-cell and B-cell flow crossmatch, and SAB on the current pre transplant sample.

Crossmatch results are summarized on page #1 and worksheets are on page #2-9

Sample dated 8-13-09 is the final crossmatch

T-cell FXM: MCS=43 (negative, cutoff:52)

B-cell FXM: MCS=432 (positive, cutoff:106)

T-cell AHG CDC: negative

Comments:

Crossmatch performed using cells from the lymph node.
 Recipient is highly alloimmunized and has multiple antibodies to HLA Class I and II as demonstrated in the pre-transplant SAB results from sample dated 8-13-09.
 Donor HLA-Type: HLA-A1, 31; B60, 55; Cw9, 10; DR4, 9; DR53; DQ7,9
 Recipient has the following DSA: DQ9; MFI=10315
 This is consistent with the positive B-cell flow crossmatch.

Summary SAB testing performed (page #10-25)

Until June 30, 2009 for liver transplants our practice was to perform a retrospective T-cell AHG-CDC crossmatch on the current (collected within 48 hours) pre transplant serum. Since July 1, 2009 our practice is to perform a retrospective T-cell AHG-CDC crossmatch, T-cell and B-cell flow crossmatch, and SAB on the current pre transplant sample. Additional SAB will be performed on post-transplant day-7, 21, 120 and one year.

Comments:

Role of DSA as defined by more sensitive solid phase assays in post liver transplant outcomes are not completely defined. There are some publications indicating that presence of preformed HLA antibodies may decrease liver allograft survival (Castillo-Rama et al., Liver Transpl. 2008 Apr;14(4):554-62), while there are others indicating that it does not affect allograft survival. In our institution we have seen 4 cases of unexplained liver allograft failure and testing for anti-HLA antibodies demonstrated very high level of DSA around that time. We are thus evaluating the role of anti-HLA antibody in liver transplant since July 1, 2009.

Summary of immediate pretransplant SAB (page# 18-21)

Sample from 8-13-09 (page #18-21) is the pre transplant sample and demonstrate multiple class I and II anti-HLA antibodies. Significant of which are as follows:

- a. HLA-DQ9: highest MFI=10,315: based on donor typing this is a DSA
- b. HLA-DQ7: highest MFI=4534: based on donor typing this is a DSA**.
- c. Antibodies to HLA-DP with high MFI

Comments:

We do not routinely type for HLA-DP antigen and thus we do not know if any of the antibodies to HLA-DP are DSA.

**Although DQ7 was interpreted as DSA, this is not the complete interpretation as the recipient himself types as HLA-DQ2, 7. This is because in the SAB tests, beads with DQB1 specificities in addition also represent DQA1 specificities. Thus a careful interpretation of all the positive and negative reactions is needed to interpret the likely antibody specificity. On review of these SAB results, one can hypothesize that this is an antibody to DQA1 specificity and not to DQB1 (DQ7) as the five beads with DQB1*0301 demonstrate MFI ranging from 92 (negative) to 4534 (positive).

Details of the beads with DQB1*0301 are as follows:

bead 051 (DQA1*0201, DQB1*0301) MFI=4534;
 bead 050 (DQA1*0301, DQB1*0301) MFI=4229;
 bead 052 (DQA1*0601, DQB1*0301) MFI=3598;
 bead 078 (DQA1*0505, DQB1*0301) MFI=954 and
 bead 077 (DQA1*0503, DQB1*0301) MFI=92

Our r-SSO typing for Class II also provides the DQA1 typing. As this method is not yet validated, we do not report the DQA1 typing. However, in this case one can use the DQA1 typing to interpret the SAB findings.

Based on the Class II r-SSO (page #27) typing the recipient is likely DQA1*0501/05/09, while the donor (page#29) is likely DQA1*0302/03.

Beads with DQA1*0302 or 0303 demonstrate high MFI as follows: bead 080 (MFI=10315), bead 081 (MFI=8209) and bead 079 (MFI=7904).

Beads with DQA1*0501 or 0505 demonstrate low MFI as follows: bead 078 (MFI=954), bead 041 (MFI=640) and bead 077 (MFI=92).

Thus it is likely that the antibody profile as defined by SAB is to the donor and not to self. However, one cannot completely rule out an autoantibody.

Summary of posttransplant SAB (page # 10-21)

Samples from 8/21/09, 8/24/09, and 8/30/09 are post transplant SAB results and demonstrate decreasing DSA MFI:

Antibody	8/13/09	8/21/09	8/24/09	8/30/09
DQ9 (bead 080)	10315	7002	5015	5045

Comments:

Our current practice is to perform SAB on pre transplant sample and on post transplant sample day 7, 21, 120, and one year. In the limited prospective cases, we find that even with a positive T-AHG CDC crossmatch and very high titer DSA pretransplant, there is very low level or no DSA by day 7 and the graft is functioning fine.

However, in this case, at day seven the liver biopsy demonstrated cellular rejection with increased liver enzymes and this was treated with pulse methylprednisolone. Thus additional SAB were performed. Along with decreasing DSA, there was improving function. A biopsy on 8/30/09 was suggestive of resolved rejection.

Comments on Procedure/Testing:

At our institution DSA MFI >4000 correlates with a positive FXM and DSA MFI values <300 correlate with a negative FXM. Between 300 and 4000 MFI, as the value increases, there is higher likelihood of a positive crossmatch. Based on these from August 10, 2009 it was decided that all MFI with values >300 will be reported to the clinicians. As our practice includes a variety of transplant programs, to simplify the process, the above cutoff is used regardless of which transplant service is ordering the test. Prior to this, the specificities as called by the software were reported as in this case.

Another reason for the presence of these antibodies could be the passive transfer from the blood products that he received.

Interpretation/Consultation:

- Negative T-cell AHG-CDC and flow crossmatch.
- Positive B-cell flow crossmatch consistent with the presence of DSA by SAB.
- Post transplant persistence of DSA.

Post Transplant Information/Comments:

Biopsy from 8/31/09 demonstrated resolved rejection. Patient was discharged on 8/31/09

and continues to do well. This case illustrates that anti-HLA antibody may have some significance in liver transplantation.

Signed:

**CASE PORTFOLIO FILE FOR HISTOCOMPATIBILITY (HLA)
LABORATORY DIRECTOR TRAINING AND QUALIFICATION**

Case Number:

1.1 patient GJ258

Category or Type of Case:

1 Related Bone Marrow Transplant

Ethnicity: Caucasian

Technology(ies)/Test(s) Used:

- HLA typing SSP
- HLA typing reverse SSO
- HLA-antibody detection by Single Antigen Beads (SAB)

Diagnosis :

High Risk Acute Myeloid Leukaemia –M5

Clinical History:

31-year-old male referred for evaluation of allogeneic hematopoietic stem cell transplant (HSCT). He was diagnosed with intermediate risk AML-M5 with no CNS involvement in August 2008 was treated with chemotherapy and was in clinical remission by October 2008. He was referred to our institute Feb 24, 2009 for evaluation for allogeneic HSCT. He was considered to have a high relapse risk and thus was recommended for HSCT. Recipient has five siblings of whom four were typed for HLA. One sibling was found to be a 6/6 match at Class I however, 2/4 allele level match at Class II. Thus a matched unrelated donor search was initiated in May 2009. The best unrelated donor available was 8/10 allele level match at HLA-A, B, Cw, DRB1 and DQB1 with one mismatch in the GVH direction only and another mismatch in the HVG or rejection direction only. Patient relapsed in June 2009 and underwent second round of chemotherapy and lumbar puncture demonstrated CNS involvement. After considerable discussion even though both the sibling and unrelated donor were 8/10 allele level match, it was decided to choose the unrelated donor over the sibling donor for transplant. The two mismatches in the sibling donor were bi-directional. Recipient was transplanted on September 9, 2009.

Number of family members available for evaluation:

Four, one sibling was typed at outside institute and was told that he was not a match.

Tests Performed

Low- medium resolution HLA Class I and II typing by reverse SSO

High resolution group specific HLA typing by SSP

Single antigen bead testing for antibodies to HLA on Luminex platform

Summary of Family Testing (page#1):

In this case HLA-typing was performed on the recipient and four siblings.

Haplotype assignment:

Four HLA-haplotypes can be assigned in this family

a. A*03, Cw*02, B*35, DRB1*1301, DRB3*0301, DQB1*0603

b. A*11, Cw*04, B*35, DRB1*0701, DRB4*0101, DQB1*0202

c. A*30, Cw*04, B*35, DRB1*1302, DRB3*0202, DQB1*0609

d. A*01, Cw*07, B*08, DRB1*17, DR52, DQB1*02

Comments:

Our practice is to initially perform HLA Class I typing on siblings and if it is a two haplotype or a 5/6 match to proceed with Class II typing. Class II typing was thus performed on Sibling-3. However, this resulted in 2/4 match, but zero mismatches at the antigen level. Thus high resolution typing was performed on sibling-3 and the recipient. To further deduce the haplotypes in the family, additional class II typing on other siblings was needed. Sibling-2 and sibling-4 are HLA identical for Class I haplotypes. Thus only sibling-2 in addition to sibling-1 was typed for Class II to deduce the haplotypes. Haplotype analysis is attached (page #2).

Summary of HLA-typing (copies of laboratory worksheets page#3-28):

Recipient demonstrates cross-over around the HLA-DRB1 region in one of the inherited haplotype and is thus haplotype (a/b)/c

Sibling 1: is a sister, haplotype a/d; 3/6 antigen match or 2/5 antigen mismatch at HLA-A, B and Cw loci. 2/4 antigen mismatch at HLA-DRB1 and DQB1. High resolution typing was not performed on this sibling, however based on the haplotype assignment, this is a 4/4 allele level mismatch at HLA-DRB1 and DQB1.

Sibling 2: is a sister, haplotype b/d; 4/6 antigen mismatch at HLA-A, B and Cw loci. 1/4 antigen mismatch at HLA-DRB1 and DQB1. High resolution typing was not performed on this sibling, however based on the low-to medium resolution typing (page#8), this is a 2/4 allele level mismatch at HLA-DRB1 and DQB1.

Sibling 3: is a sister, haplotype a/c; at least one haplotype antigen *match* at HLA-A, B, Cw, DRB1 and DQB1. She is 6/6 allele level match at HLA-A, B and Cw loci. However, the sibling is 2/4 allele level mismatch at HLA-DRB1 and DQB1 loci.

Sibling 4: is a brother, haplotype b/d; only Class I typing was performed and is a two haplotype mismatch at HLA-A, B and Cw.

Comments:

Class II typing on sibling-3 demonstrated that she is homozygous at HLA-DRB1 (HLA-DRB1*13) and DQB1 (HLA-DQB1*06) and thus a 2/4 match but zero mismatch with the recipient. Both antigen mismatches are in the GVH direction on low resolution. High resolution typing was performed and this demonstrated that sibling-3 is heterozygous at both loci: HLA-DRB1*1301, 1302 and HLA-DQB1*0603, 0609. This meant that these are bi-directional mismatches with the recipient.

Interpretation/consultation:

Recipient has a crossover around the DRB1 region resulting in an unusual haplotype. Sibling-3 is 7/9 allele level match at HLA-A, B, Cw, DRB1 and DQB1. Both the sibling and recipient have one allele at HLA-B locus. An unrelated search is indicated.

NMDP Unrelated Search Evaluation (page # 29-40):

NMDP search indicates it is very unlikely that a 10/10 HLA (A,B, Cw, DRB1 and DQB1) matched donor will be identified.

Based on the NMDP match grade/calculation, five potential donors (indicated by the star sign on page #29, 30 and 38) that will be at least 6/8 match at HLA-A,B, Cw and DRB1 were identified and samples requested for evaluation in May 2009.

An official search consult from NMDP was also requested.

For reference recipient HLA typing:

HLA-A	HLA-B	HLA-DRB1	HLA-DQB1	HLA-Cw
0301, 3002	3501, -	0701, 1302	0202, 0609	0202, 0401

Evaluation of NMDP donor typing(copies of laboratory worksheets page#41-55)::

Samples were requested from five donors. However, only two were available. Typing is summarized below: Row titled NMDP is the typing available in NMDP database.

NMDP Donor-1 (0258-5777-2):

	HLA-A	HLA-B	HLA-DRB1	HLA-DQB1	HLA-Cw
NMDP	03, 30	35	0701, 13	NA	NA
Our typing	03, 30	35	07, 13	02, 05	04, -

Comments:

Two antigen mismatch on low-medium resolution.

Additionally, from the low to medium resolution typing results the donor is not homozygous for B*35 at the allele level. This donor is likely B*35XX1, 3508. Also the donor is likely DRB1*1301 while the recipient is DRB1*1302.

No additional testing was performed.

NMDP Donor-2 (PVAD36987)

	HLA-A	HLA-B	HLA-DRB1	HLA-DQB1	HLA-Cw
NMDP	03, 30	35	07, 13	NA	NA
Our typing	03, 30	35	07, 13	02, 06	04, -
Low res					
High res	0301, 3002	3501, 3503	0701, 1302	0202, 0609	0401, 0401

Comments:

Low-med resolution: One antigen mismatch (HLA-Cw) in GVH direction
 High resolution: One allele (HLA-B) mismatch in HVG direction and 1 antigen (HLA-Cw) mismatch in GVH direction.

NMDP Unrelated Cord Search Evaluation (page #59-66):

As it was unlikely to find a 10/10 match, NMDP search for umbilical cord was also performed. Based on NMDP match grade/calculation there were no umbilical cord units that were 4/6 match at HLA-A, B and DRB1 with a TNC $> 2.5 \times 10^7$ cells.

Comments:

Our institution initiated the umbilical cord transplant program this year. If a cord was to be selected, this recipient would have been our first case.

NMDP Search Consult Summary (page #56-58):

Search consult also indicated that the donors selected by us were good choices and it was unlikely to find a 10/10 match. However, there was a potential to get 9/10 match if one were to mismatch at HLA-B.

Comments:

It was likely that even in this scenario one cannot rule out additional mismatches. Additionally as the recipient is homozygous at HLA-B, this will mean a mismatch in HVG direction. As the patient by now had a second relapse, clinical decision was made to go with NMDP donor-2.

Donor Selection:

It was evident that in this case it is going nearly impossible to find a 10/10 match for matched unrelated donor or alternatively a better cord. We were now faced with two potential donors

- a. NMDP donor-2: who is 8/10 match with one antigen mismatch in the GVH direction and other allele mismatch in the HVG direction.
- b. Sibling donor-3: who is a 6/6 match at HLA-A,B and Cw. At low resolution she was 2/4 match and it seemed like she was homozygous at HLA-DRB1 and DQB1 with both mismatches in GVH direction. However, high resolution demonstrated that sibling 3 was heterozygous at HLA-DRB1 and DQB1 and both the mismatches were bidirectional

Considering all this, it was decided that the unrelated donor will be a better option. Recipient received transplant from the unrelated donor.

Evaluation of Single Antigen Bead testing (page 67-74):

Performed before the transplant on 8/18/09 sample and demonstrated very low level anti-HLA antibodies. None of which were donor specific (DSA). SAB was performed again post transplant on October 14 as there was no engraftment. Once again there were no DSA.

Interpretation/Consultation:

Considering the extensive search conducted, it is unlikely to find a 10/10 matched unrelated donor.

NMDP donor-2 is the best option in this scenario.

Additional Information/Comments:

Patient underwent a myeloablative HSCT on September 9, 2009. His post transplant immunosuppression included sirolimus and methotrexate for GVHD. At day +35 there was no engraftment and the patient had neutropenic fevers, VRE colonization.

SAB was performed again but demonstrated low level of anti-HLA antibodies and no DSA.

Clinically it was decided to request additional cells from the NMDP donor. Also the option of second transplant was discussed.

For this option; sibling donor-3 was reconsidered but once again the decision was to not proceed with this.

Recipient received second transplant from the same NMDP donor. The conditioning regimen was myeloablative and included alemtuzumab (Campath). At day 30 post second transplant the recipient was transfusion independent and chimerism study demonstrated 100% donor cells

Signed:

**CASE PORTFOLIO FILE FOR HISTOCOMPATIBILITY (HLA)
LABORATORY DIRECTOR TRAINING AND QUALIFICATION**

Case Number:

2.1 patient YL910

Category or Type of Case:

2 Unrelated Bone Marrow Transplant

Ethnicity: Native American Indian

Technology(ies)/Test(s) Used:

- HLA typing SSP
- HLA typing SSO
- HLA-antibody detection by Single Antigen Bead (SAB)

Diagnosis:

Acute Myeloid Leukemia (AML)

Clinical History:

This is a 52-year-old female for evaluation of allogeneic hematopoietic stem cell transplant (HSCT). She was diagnosed with undifferentiated AML in January, 2009 and was treated with chemotherapy. Her subsequent biopsy demonstrated some residual disease and thus she underwent second re induction chemotherapy. Subsequent to this there has been no marrow reconstitution and her stay has been complicated with neutropenic fevers and bacteremia. Patient is now transfusion dependent and was recommended for HSCT. Recipient's two siblings were typed for HLA but neither were a match. Thus a matched-unrelated donor (MUD) search was initiated in May, 2009. There were no potential 10/10 match MUD donors. The option of an umbilical cord transplant was also considered. While awaiting the donor samples, patient developed VRE and breast abscess which needed surgical intervention. She was also informed that her insurance will not cover cord transplant. Even though a 9/10 matched MUD was identified, with the ongoing infectious complications patient decided against HSCT.

Number of family members available for evaluation:

Two siblings, both were typed.

Tests Performed

Low- medium resolution HLA Class I and II typing by reverse SSO

Group specific high resolution HLA typing by SSP

SAB testing for antibodies to HLA

Evaluation of Family Testing:*Haplotype assignment:*

Four haplotypes can be assigned in this family:

a: A*02, Cw*04, B*35

b: A*24, Cw*07, B*39

c: A*24, Cw*15, B*51

d: A*31, Cw*07, B*39

Class II segregation is not possible as our practice is to initially perform Class I typing on siblings and if it is a 5/6 match or two haplotype match to proceed with Class II typing.

Summary of Class I typing (page# 1):

Sibling-1: is haplotype b/c: one haplotype mismatch at HLA-A, B and Cw loci

Sibling-2: is haplotype b/d: two haplotype mismatch at HLA-A, B and Cw loci

Comments:

When evaluating family members to be potential donors for HSCT, initially only HLA Class I typing is performed. If the potential related donor is not a two haplotype match, additional typing is not performed. This is because our institution does not perform one haplotype match transplants.

Interpretation:

One sibling is one haplotype mismatch while the other is a two haplotype mismatch.

Initiation of a MUD search is recommended.

NMDP Unrelated Search Evaluation:

NMDP search indicates it is very unlikely that a 10/10 HLA (A, B, Cw, DRB1 and DQB1) matched donor will be identified due to the recipient having uncommon haplotypes. The likely haplotypes are:

A: A*0201, B*3501, Cw*0404, DRB1*0407, DQB1*0302 and

B: A*2402, B*5101, Cw*1502, DRB1*1501, DQB1*0602

Haplotype A is uncommon. Haplotype B is also an uncommon haplotype and ranks 819 in the people of European descent in the US population (bioinformatics.nmdp.org).

Based on the NMDP match grade/calculation, seven potential donors (indicated by the star sign on page #) that may be at least 6/8 match at HLA-A,B, Cw and DRB1 were identified and samples requested for evaluation in May, 2009.

For reference recipient HLA typing:

HLA-A	HLA-B	HLA-DRB1	HLA-DQB1	HLA-Cw
0201, 2402	3501, 5101	0407, 1501	0302, 0602	0404, 1502

Evaluation of NMDP donor typing (page #58-66):

Samples were requested from seven donors indicated by star sign on page #58, 60 and 61); however, only five were available. Typing is summarized below: Row titled NMDP is the typing available in NMDP database.

NMDP Donor-1 (5082-6185-6):

	HLA-A	HLA-B	HLA-DRB1	HLA-DQB1	HLA-Cw
NMDP	02, 24	35, 51	04, 15	03XX, 06	04,15
Our typing Low res	02, 24	35, 51	04, 15	07, 06	04,15
High res	0201, 2402	3503, 5101	0407, 1501	0301, 0602	0401, 1502

Comments:

Low-med resolution: One antigen mismatch (HLA-DQB1).

High resolution: Two allele (HLA-B and Cw) mismatch and one antigen (HLA-DQB1) mismatch.

NMDP Donor-2 (5019-0423-9):

	HLA-A	HLA-B	HLA-DRB1	HLA-DQB1	HLA-Cw
NMDP	0201, 2402	3503, 5101	0407, 1501	0301, 0602	04, 15
Our typing Low res	02, 24	35, 51	04, 15	07, 06	04,15
High res	0201, 2402	3503, 5101	0407, 1501	0301, 0602	0401, 1502

Comments:

Low-med resolution: One antigen mismatch (HLA-DQB1).

High resolution: Two allele (HLA-B and Cw) mismatch and one antigen (HLA-DQB1) mismatch.

NMDP Donor-3 (0016-3974-9):

	HLA-A	HLA-B	HLA-DRB1	HLA-DQB1	HLA-Cw
NMDP	s2, 24	s35, 51	s4, 15	s3, 6	NA
Our typing Low res	02, 24	35, 52	04, 15	08, 06	04,12
High res	ND	ND	ND	ND	ND

Comments:

Low-med resolution: Two antigen mismatch (HLA-B and Cw). NMDP serologic typing of the donor was HLA-B51. However, molecular typing of the donor was HLA-B*52. This is because serologic typing is prone to cross reaction between B51 and B52 which are splits of B5.

Additionally, from the low-to-medium resolution typing results, the donor is likely A*2403, DRB1*0402 and DQB1*0601 while the recipient is A*2402, DRB1*0404 and DQB1*0602 (page#25-27). This will mean a 5/10 match. Thus no additional testing was performed.

NMDP Donor-4 (0007-5659-3):

	HLA-A	HLA-B	HLA-DRB1	HLA-DQB1	HLA-Cw
NMDP	s2, 24	s35, 51	s4, 15	s8, 6	NA
Our typing Low res	02, 24	35, 51	04, 15	08, 05	04,15
High res	0201, 2402	3501, 5101	0401, 1513	0302, 0502	0402, 1502

Comments:

Low-med resolution: DQB1 antigen mismatch.

High resolution: 3 allele and 1 antigen mismatch.

HLA-Cw*1513 is relatively uncommon allele. However, this was one of the possible alleles on low to medium resolution and high resolution confirmed it, specifically the positive reaction in lane #3 of the HLA-Cw gel (page# 33).

NMDP serologic typing of the donor was HLA-DQ6. However, molecular typing of the donor was HLA-DQB1*05 which was also confirmed on high resolution. Considering the common DRB1-DQB1 linkage disequilibrium: DRB1*04 is in close linkage with DQB1*08, while DRB1*15 is in close linkage with DQ6. However molecular typing confirms the less common (1.2% in Caucasian) DRB1*15 linkage with DQB1*05.

Additionally, as the original NMDP typing was done by serology, one can speculate that the discrepancy between DQ6 by serology versus DQ5 by molecular typing is likely due to cross reaction. (DQ5 and DQ6 are splits of DQ1).

NMDP Donor-5 (0361-7374-8):

	HLA-A	HLA-B	HLA-DRB1	HLA-DQB1	HLA-Cw
NMDP	0201, 2402	3501, 5101	0407, 1501	0302, 0602	04,16
Our typing Low res	02, 24	35, 51	04, 15	03, 06	04,16
High res	0201, 2402	3501, 5101	0407, 1501	0302, 0602	0404, 1602

Comments:

Low-med resolution: One antigen mismatch (HLA-Cw).

High resolution: One antigen (HLA-Cw) mismatch.

NMDP Unrelated Cord Search Evaluation (page #67-70):

As it was unlikely to find a 10/10 match, NMDP search for umbilical cord was also performed. Based on NMDP match grade/calculation there were umbilical cord units that were 4/6 match at HLA-A, B and DRB1 with a TNC > 2.5 x 10⁷ cells.

Comments:

Our institution initiated the umbilical cord transplant program this year. If a cord was to be selected, this recipient would have been our first adult case. However, the recipient's insurance does not cover umbilical cord transplant. Additionally, the recipient was less likely to pursue HSCT. Thus no additional investigations were carried out.

Donor Selection:

It was evident that in this case it is going to be an extremely difficult to find a 10/10 match for MUD. Of the donors evaluated only one donor (donor-5) was a 9/10 match at HLA-A, B, Cw, DRB1 and DQB1. The other four donors were at least 7/10 match at HLA-A, B, Cw, DRB1 and DQB1.

Considering the clinical scenario, only donor-5 is the optimal choice.

Evaluation of Single Antigen Bead testing (page #71-76):

Performed on 2/23/09 sample and demonstrated multiple Class I and Class II anti-HLA antibodies, some of them with high MFI.

Comments:

At our institute DSA MFI >4000 correlate with a positive FXM and DSA MFI values <300 correlate with a negative FXM. Between 300 and 4000 MFI as the value increases there is higher likelihood of a positive crossmatch. Based on these from August 10, 2009 it was decided that all MFI with values >300 will be reported to the clinicians. As our practice includes variety of transplant programs, to simplify the process, the above cutoff is used regardless of which transplant service is ordering the test. Prior to this, the specificities as called by the software were reported.

Interpretation/Consultation:

Considering the extensive search conducted, it is unlikely to find a 10/10 matched unrelated donor. NMDP donor-5 is the best option in this scenario.

Additional Information/Comments:

While awaiting the workup, patient developed breast abscess, VRE and continued to be transfusion dependent. Patient decided to not pursue HSCT.

Signed: