POLICIES AND PROCEDURES FOR
AMERICAN FOUNDATION FOR DONATION AND TRANSPLANTATION (AFDT)
PROFICIENCY TESTING PROGRAM
Version 2013.000

POLICIES

Oversight Policies

Policy 1)   The Proficiency Testing Committee of AFDT oversees the Proficiency Testing Program (PT). This committee is composed of program users from both member and non-member. The Director of Operations serves as the Administrative Coordinator. The program also has Technical and Database Managers.

Policy 2)   The policies of the PT program are established by the Proficiency Testing Committee of AFDT. Financial policies and membership issues must be approved by the AFDT Board of Directors.

Policy 3)   **Remedy for receipt of inadequate sample or non receipt of sample**. Upon receipt of sample, it is the responsibility of the receiving lab to inspect the shipment for any damage. If a sample is received broken or in otherwise unsuitable condition, it is the responsibility of the receiving laboratory to notify the Administrative Coordinator by phone, fax, or email within 1 (one) hour of sample receipt. Documentation of the time of receipt of samples in the laboratory may be required. If the receiving laboratory does not provide notification of sample damage within 1 hour of receipt of samples in the laboratory, the receiving laboratory will be graded as incorrect due to a lack of reported results.

If notified within 1 hour of receipt of the shipment in the laboratory, the Administrative Coordinator will have 2 options at his/her discretion: 1) if feasible, replace the original sample or 2) not count the missing cell or serum as a miss. If available, serum samples for the crossmatch exchange may be replaced by the sending laboratory within 24 hrs. The serum samples should then be tested for anti HLA antibody and crossmatched with cells received in the underline original shipment. Additional cell and serum samples will not normally be available, except for the reasons described above.

For laboratories in the continental US, if the samples are not received in the laboratory by 4 PM according to the shipping schedule, the laboratory must notify the Administrative Coordinator by 4:30 PM so that tracking of the samples by FEDEX/UPS may be initiated. If samples cannot be delivered as outlined above, the options for remedy will be utilized. AFDT cannot assure overnight delivery of samples outside of the continental US.

Policy 4) **Reporting of grades**. Each laboratory will receive a final graded report. Grades should be kept confidential, since they may be used for laboratory accreditation.

Grades will be reported to federal regulatory or state agencies only at the written request of the laboratory. Those laboratories that want their results directed to any accrediting or other oversight agencies must provide all pertinent information to AFDT on an annual basis. It is the responsibility of the laboratory to notify AFDT of any changes in laboratory status. As of 1999, AFDT Proficiency Testing has been accepted by the College of American Pathologists (CAP) for the purposes of the Laboratory Accreditation Program. Laboratories wishing to have AFDT PT results sent to CAP must provide AFDT with written consent and the laboratory's Laboratory Accreditation Program (LAP) number.

Entry Instructions & Grading Policies – Cell Typing (See Appendix D)

Entry Instructions & Grading Policies – PRA (See Appendix E)

Entry Instructions & Grading Policies – Crossmatch (See Appendix E)

Data Submission and Turnaround Policies

Policy 1) **Attestation Statement**: Each laboratory must sign an attestation statement certifying that all proficiency testing is performed in the same manner as clinical samples. The original signed version of this document is kept by the lab for presentation to official agencies, and a copy with typed names of testing personnel and Director, or designee, who reviewed the work will be sent to AFDT via fax, mail or email.

Policy 2) Each laboratory is responsible for timely and proper submission of data to the AFDT Proficiency Testing Program.

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Policy 3)   Lab data submission results are due 1 month after shipment. Reports are sent to labs within 3 weeks after results received. CAP and state reports are sent 6 weeks after lab data submission of results.

Financial Policies

Policy 1)   The fee structure for the AFDT Proficiency Testing Program will be determined by the PT Committee with advice from the AFDT Administrative Coordinator and Controller as to expenses and revenue. Laboratories requesting participation in only the Cell Exchange or the Serum Exchange will be billed at a different rate than the full exchange. An additional fee will be assessed for any shipment made outside the continental US.

Policy 2)   Reimbursement to the send-out laboratories will be determined by the Proficiency Testing Committee, with advice from the AFDT Administrative Coordinator and Controller as to expenses and revenue. The rate of reimbursement will be on a per lab basis, that is, the send-out laboratory will receive a set fee for each set of samples that is sent out to a lab.

PROCEDURES

Send-out procedures

   Send-out dates for the AFDT Proficiency Program are published on the AFDT website and are also mailed/emailed to participants at the beginning of each testing year. **Send-out laboratories** will be selected by the Proficiency Testing Committee of laboratories who express a willingness to serve as such. Each of these laboratories will normally be responsible for one or more send-outs per year, on a schedule to be determined by the PT Committee and Administrative Coordinator. The PT Administrative Coordinator will provide send-out labs with a list of subscriber labs to which samples should be sent. The PT Administrative Coordinator will 1) provide a cover letter, address and sample labels, and all other necessary information for the test samples to be shipped, 2) send a letter to each subscribing laboratory notifying them of the date of sample send-outs. Shipments must be sent to US laboratories by overnight carrier, to reach the laboratory by 10AM the next day. Samples sent outside of the continental US must be sent by the most rapid carrier service readily available. Blood samples must be packed securely to avoid breakage and must conform to all federal, state, and shipper's regulations.

Cell Exchange: Cells will be selected from pre-typed, willing donors, who are known to provide a cell yield suitable to perform HLA typing.   Each send-out will consist of blood from 5 donors. Blood should be drawn into ACD, with each subscribing lab receiving a 10 ml tube of whole blood for each sample.

Crossmatch Exchange: A 10cc tube of whole blood in ACD from each of 2 pre-typed willing donors will be sent to each participating lab for crossmatching.   The HLA type of the cells used for crossmatching will be provided by the sending lab.   In addition, 5 serum samples (1.0 ml each) which have been well characterized for anti-HLA antibody by cytotoxicity, flow and ELISA Luminex will be sent for antibody analysis and crossmatching. Blood should be drawn into ACD, with each subscribing lab receiving a 10 ml tube of whole blood for each sample.

Data Entry

AFDT provides a data entry MS-ACCESS application to each participating lab. The participating lab personnel uses this application to record their results. After adding their results, the application provides a way for the user to save the results to an Excel file that is emailed back to the AFDT Database Manager. Each laboratory is responsible for proper data entry. Data entry errors/typos made by the lab will be considered as misses. Upon receipt of the Excel file, the AFDT Database Manager is responsible for making sure the records are appended to the AFDT Grading Program application for inclusion in the PT for the event being processed. The Excel file sent to the Database Manager from the lab will be saved, at a minimum, until the grading and lab challenge resolution period has expired.

Data Analysis

Data analysis/grading is the responsibility of the AFDT Database Manager with oversite and final approval from the AFDT PT Committee. The AFDT PT Database Manager is responsible for the maintenance and upgrades of the AFDT PT Data Entry and the AFDT PT Grading Application programs. Data analysis/grading will be performed in a manner that conforms to the policies and procedures set forth by the AFDT PT program.

Data to PT Committee Chairs

The AFDT Database Manager is responsible for providing the members of the PT Committee with copies of the graded reports for their review prior to sending graded reports to the participating labs. The graded results are saved in pdf formatted reports and transmitted by email to each Chair. The Chairs will conduct their review and submit any changes, comments, or acceptance of reports to the AFDT Database Manager. Any

changes or comments from the Chairs will be incorporated into the graded reports as needed before the final reports are sent to the lab.

The graded reports, along with consensus and challenge information will be provided by the AFDT PT Database Manager to the Administrative Coordinator and/or designated report writers for his/her use in preparation of a final summary report. The final summary report will summarize the data submitted and provide an educational discussion of the results designed to help the laboratory interpret the data and apply its findings in the future.

College of American Pathologist (CAP)/STATE

Send-out dates. A list of send-out dates and the dates by which CAP will receive AFDT results on the send-outs must be sent to CAP by June 30 of the year prior to the send-out.

AFDT proficiency for CAP participants.
A list of subscribers who wish AFDT results be sent to CAP is requested by December 1. AFDT will send the names of all CAP participants from the previous year at this time unless instructed otherwise by the laboratory. New enrollees or those who newly opt to have data sent to CAP this year must be received by CAP at least 2 months before the date CAP is required to receive results (to have data for all surveys accepted by CAP, the laboratory must ensure that AFDT has received authorization for data by February 15th).

Use of AFDT data for CAP accreditation requires that the laboratory provide written authorization to AFDT to transmit these data. The laboratory must ensure that AFDT has received authorization for PR results to be released to CAP by February 15th. Submission of the laboratory's CAP LAP number to AFDT will also be required for data to be transmitted to CAP.

Reporting to State agencies
At the time of registration for AFDT proficiency testing, laboratories wishing the proficiency testing results to be sent to their State agencies (for CLIA purposes) must fill out the appropriate request which gives AFDT authorization to release the results.

Results will be sent to the State agencies after the challenge period has expired following each send-out.

APPENDICES

Appendices should be reviewed annually by the Proficency Testing Committee and updated to reflect current fees, payments, sendout labs, major miss antigens, and analytes offered.   These changes must be approved by the AFDT PT Committee.

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APPENDIX A - Analytes offered – 2013

Results entered for the following analytes will be graded and results may be sent to CAP if so requested by the laboratory. Laboratories participating in the AFDT survey should provide data for all categories for which testing is performed in the laboratory.

HLA Typing
HLA-Class I (HLA-A,B,C) – Low Resolution
HLA-Class I (HLA-A,B,C) – High Resolution
HLA-Class II (HLA-DR, DQB1) – Low Resolution
HLA-Class II (HLA-DR, DQB1) – High Resolution

HLA A (antigen level resolution) (allele level)
HLA B (antigen level resolution) (allele level)
HLA DR (antigen level resolution) (allele level)
Antigen level typing of Cw, DQ, DR51,52,53
Reference for antigen equivalents:
Schreuder, G.M.T., C.K. Hurley, S.G.E. Marsh, M. Lau, M. Maiers, C. Kollman, and H. Noreen. Report on serological equivalents of HLA alleles.   The HLA dictionary 1999: A summary of HLA-A, -B,-C, -DRB1/3/4/5, -DQB1 alleles and their association with serologically defined HLA-A, -B,-C, -DR and -DQ antigens. Tissue Antigens 53: 409-437, 1999.

Crossmatching
CDC-T
CDC-B
AHG-T
AHG-B
No wash crossmatch - T
No wash crossmatch - B
Wash crossmatch - T
Wash crossmatch - B
AHG crossmatch - T
AHG crossmatch - B
Flow crossmatch (IgG) - T
Flow crossmatch (IgG) - B
Solid Phase XM-Class I and Class II

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HLA Antibody
Class I antibody-cytotoxicity/AHG
Class II antibody-cytotoxicity
Solid phase antibody testing:
Class I antibody screen (present or absent)
Class II antibody screen (present or absent)
Class I antibody specificity-phenotypic panel
Class I antibody specificity-single antigen panel
Class II antibody specificity-phenotypic panel
Class II antibody specificity-single antigen panel

Results for the following analytes are currently not graded, but results are available to CAP as ungraded analytes if so requested by the laboratory and to participating laboratories for self assessment.

HLA typing:

Bw4/Bw6
DR51,52,53 (DRB3/4/5)
DQA1 – low resolution
DQA1 – high resolution

DPB1 – low resolution
DPB1 – high resolution

Class I antibody specificity
Class II antibody specificity

Additionally, data is collected in the following categories and is made available to the participating laboratories.
- Data on PRA by method
- Data on Crossmatch by more specific methods

APPENDIX B – <u>download from UNOS web site</u>. Sample is below as of September, 2013.

http://optn.transplant.hrsa.gov/policiesAndBylaws/policies.asp

keyword search: equivalences (UNOS appendix 3A)

HLA Antigen Values and Split Equivalences

HLA A, B, and DR Matching Antigen Equivalences					
PATIENT A LOCUS ANTIGEN	EQUIVALENT DONOR ANTIGEN(S)	PATIENT B LOCUS ANTIGEN	EQUIVALENT DONOR ANTIGEN(S)	PATIENT DR LOCUS ANTIGEN	EQUIVALENT DONOR ANTIGEN(S)
1	1	5	5,52,53,78	1	1,103
2	2,203,210	7	7,703	2	2,15,16
3	3	8	8	3	3,17,18
9	9	12	12	4	4
10	10,26,34,66,*6601,*6602	13	13	5	5,11,12
11	11	14	14,64,65	6	6,13,14,1403,1404
19	19,74	15	15,75,76,77,*1304	7	7
23	23	16	16,*3905	8	8
24	24,2403	17	17,58	9	9
25	25	18	18	10	10
26	26,*6601	21	21,4005,*1304	11	11,5
28	28,68,69	22	22,54,*8201	12	12,5
29	29	27	27	13	13,6
30	30	35	35	14	14,6,1403,1404
31	31	37	37	15	15,2
32	32	38	38	16	16,2,15
33	33	39	39,3901,3902,*3905	17	17,3,18
34	34,*6602	40	40,61,81	18	18,3,17
36	36	41	41	103	103,1
43	43	42	42	1403	1403,14,6
66	66,*6601,*6602,10	44	44	1404	1404,14,6
68	68,28	45	45	** 99	(No equivalent)
69	69,28	46	46		
74	74,19	47	47		
80	80	48	48		
203	203,2	49	49		
210	210,2	50	50,4005		
2403	2403,24	51	51,5102,5103		
*6601	*6601,66,10,26	52	52,5		
	*6602,66,10,34	53	53,5,5102		
*6602	(No equivalent)	54	54,22		
		55	55		
** 99		56	56		
		57	57		
		58	58		
		59	59		
		60	60		
		61	61,40		
		62	62		
		63	63		

		64	64,14		
		65	65,14		
		67	67		
		70	70,71,72		
		71	71,70		
		72	72,70		
		73	73		
		75	75,15		
		76	76,15		
		77	77,15		
		78	78,5		
		81	81,7,40,60,61,48		
		703	703,7		
		*0804	*0804,8		
		*1304	*1304,15,21,49,50		
		2708	2708,27,7		
		3901	3901,39		
		3902	3902,39		
		*3905	*3905,16,39		
		4005	4005,21,50		
		5102	5102,51,53		
		5103	5103,51		
		*8201	*8201,45,22,54,55,56		
		** 99	(No equivalent)		

* Indicates an allele; may not have a WHO-approved serologic specificity

APPENDIX C

Financial

Payment to send-out laboratories, $125/lab for the cell typing send-outs and $150/lab for the XM/PRA send-outs.

Fees from laboratories

     PT program
         $1,000 per year AFDT member lab
         $1,700 per year non member lab
     Cell exchange only
         $750 per year AFDT member lab
         $1,200 per year non member lab
     Serum exchange only
         $750 per year AFDT member lab
         $1,200 per year non member lab
     Additional cell or serum exchange
         $350 for AFDT member labs
         $450 for non member labs

Expenses of screening sera for send-out should be billed by the provider laboratory to AFDT.

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APPENDIX D

2013 HLA Typing Survey

Instructions

Entry for High resolution typing will require insertion of colon as described below .

Survey Description:

The AFDT HLA Typing Survey is designed to evaluate a laboratory's ability to accurately identify HLA antigens present on unknown cells.

There will be 3 sendouts per year with 5 samples per sendout.

The same sendout will serve as the cells for:

Serology Testing, Class I and Class II
Molecular Testing, Class I and Class II Low resolution
Molecular Testing, Class I and Class II High resolution
Antigen Level Results – translation of Molecular Typing to Antigen name

Software for HLA Typing and XM/PRA Proficiency Testing

Application software is provided by AFDT for the HLA Typing and XM/HLA Ab surveys. You should have recently gotten the latest version for 2012 and forward. If you do not have this software, please contact Arlene Skinner at skinner@seopf.org.

When you receive the software, it will be a zipped file. Save the zipped file to your desktop. Then right click on the icon and choose "Extract All". Place the unzipped AFDT folder on the C drive. Remove the zipped folder to your Recycle bin. You can open the AFDT folder, right click on the application icon and choose "Make Shortcut" to add a shortcut to your desktop.

The first time you use the application software, you will need to enter your laboratory name and lab code. The lab code was provided by AFDT.

Choose **HLA Typing** to enter HLA typing results. After the results have been entered, you can print the results for review and to keep for your records. Then you can directly e-mail the results

back to the by Clicking "Create e-Mail File for Sending" on the HLA Typing menu page. (Some systems have firewalls that may prevent direct e-mail. In this case, select "Export files for sending" to save the files to your hard drive in the AFDT folder. You can then e-mail the file to molangan@sc.rr.com.)

For new users there are detailed application instructions sent with the software. Please review before loading and using the software the first time. For any software-related problems, please contact Marilyn Langan at molangan@sc.rr.com.

Results Entry for HLA Typing Survey:
The software has four Tabs for data entry: Antigen results from Molecular typing, Serology results, Low Resolution DNA typing, and High Resolution DNA typing. The Antigen results should be filled in only by labs performing molecular typing as this will assess the laboratory's ability to "translate" the DNA result into its Antigen equivalent, as required for UNET entry for Solid Organ transplantation. Results derived from serological typing should only be entered under the Serology Tab. These results should be based only on serology testing and will be graded separately. The Low Resolution results are provided for educational purposes only (except for DQA1, DPA1, and DPB1) since the Antigen level typing is graded. High resolution typing is also graded. High resolution results should only be entered by labs using methods intended for high resolution and not when a high resolution result is occasionally obtained using a low resolution method. Results for DQA1, DPA1, and DPB1 can be entered and will be available as a separate report that will be posted on-line. DQA1, DPA1, and DPB1 will be graded separately and results are made available for self assessment. At least 8 responses are required for grading; otherwise self-assessment is needed.

Please notice that the default is NT (not tested).

NT: Leave NT in the box only if you did not test for a particular locus.

No 2nd Antigen: If you typed for the locus, but only found one antigen, then put the antigen in the first box and choose "No 2nd Ag" in the second box.

ND: Not detected: For DRB3/4/5, enter ND if the locus was tested, but no antigen was detected for that locus.

Report high resolution ONLY if a method was used which is designed for high resolution typing. If high resolution was obtained for one antigen, but not for the other antigen for a particular locus, the lab may report the high resolution result for the first locus and leave NT for the second antigen

since the lab was unable to determine high resolution for the second antigen. Report the most probable high resolution typing and indicate in comments the ambiguities obtained with high resolution.

Antigen Results:
Enter the Antigen level result for the loci typed by molecular methods. Molecular results need to be "translated" to the serological equivalent. For antigens that do not have a serological equivalent, enter the first two digits of the DNA result.   All Cw's will be entered as an antigen even if there is not a serological equivalent (Ex. Cw12).
The results under the Antigen Tab should mimic what a laboratory would enter into UNET for solid organ typing. For Bw4 and Bw6, choose (P)os or (N)eg, or Null for each. For DR51, 52, 53, choose from the dropdown menu. If only one antigen found for DR51,52,53B3/4/5, choose (BL) "No second antigen" for the second result. If no antigens are detected for DR51, 52,53, enter (ND) "No antigen detected" for both the first antigen and BL "No second antigen" for the second result.

You must use the Antigen name, not the DNA name the Antigen results. (Ex: A2, not A*02; B62, not B15; Cw9, not Cw*03; DR17, not DR 03, etc.)

Serology Results:
Enter serology results derived from using serological trays under the Serology Tab. Serology only labs should **NOT** enter results under the Antigen Tab. Serology results will be graded separately. Enter the serological type derived from serological typing trays. If your laboratory does not use a serology (CDC) method, leave this table as it is with the NT's in each field. For Bw4 and Bw6, choose (P)os or (N)eg for each. For DR51, 52, 53, choose from the dropdown menu. If only one antigen found for DR51,52,53, choose "No second antigen" for the second result. If no antigens are detected for DR51, 52,53, enter "ND" - No antigen detected for both the first antigen and "BL" – No second antigen for the second result.

You must use the Antigen name, not the DNA name the Serology results (Ex: A2, not A*02).

Cw typing by serology is for educational purposes only and is not included in the grading for serology results.

Molecular typing- Low resolution:
Enter the first 2 digits of the molecular typing into the table for low resolution typing. **Be sure to also put the "antigen" translation under the Antigen tab**. For Bw4 and Bw6, choose (P)os,

(N)eg, or Null for each. For DRB3, DRB4, and DRB5, choose (P)os, (N)eg, or Null for each.

(**Use only DNA nomenclature in the DNA Low resolution typing results**! Serology nomenclature will be seen as a discrepancy if entered for the DNA results!)

Example: B*1501/17/43++ should be entered in this table as B**15**, NOT B**62**.
             DQB1*0301/09/+ should be entered as DQB1***03**, NOT DQ**7**

The antigen level names for the Low resolution results will be put into the Antigen Table and graded as such. When entering results in the Antigen Table, include all the Cw antigens (including **Cw12,13,14,15,16,17,18**) even though they were not previously recognized using serological typing.

Molecular typing – High resolution
Laboratories must enter high resolution typing using the new nomenclature (with colon). Please put a colon between the antigen portion of the result (usually after the first two digits) and the allelic portion of the result (the last two or more digits) Ex. A*02:01

Enter the high resolution type.   Enter the most probable type in the case of ambiguous combinations, using the list of common and well-documented alleles as a guide. You may put the other possible combinations in the comment box. There will be space available in the result entry box to enter N for null alleles, etc.

Laboratories should resolve the following ambiguous combinations:

1. When both of the alleles appear on the common and well-documented list (CWD) in more than one potential combination of alleles. A link to the NMDP rare alleles list can be found at : http://bioinformatics.nmdp.org/HLA/Rare_Allele_Lists/Biannual_Rare_allele_Lists.aspx

             OR

2. When there is no combination that has both alleles on the CWD list and there are more than one possible combination in which only one allele appears on the CWD list.

Note: For PT purposes, a laboratory does NOT have to resolve ambiguous combinations when there is only one possible result in which both alleles are on the common and well-documented list of alleles.

The rare ambiguous combinations that were not ruled out should be listed in Comments.

For DRB3,4,and 5 (**high resolution**): there will be two fields for each. If the locus was typed at high resolution, enter the high resolution results in the boxes.
- If there was only one allele for the locus, put the allele in the first box and choose BL – "No 2nd Ag" in the second field.
- If the loci were tested, but no DRB3/4/5 were detected, place ND – "No Ag detected" in the first field and BL – "No second antigen" in the second field.
- If only low resolution was performed, leave NT in both fields.

Special Notes on Result Entry:

- Enter the smallest number first for each locus
- Bw4 and Bw6 will be reported as (P)os or (N)eg
- A choice of ND - "No antigen detected" is provided in the dropdown menu and can be used for high resolution DRB3/4/5 results when the antigen is not present at all.
- If only one antigen or allele was found for a locus, enter the result in the first field and choose "No 2nd Ag" in the second field. Do not leave NT in the field if testing was done for the locus.
- The antigen "split" must be entered whenever possible for the Antigen results. Parent antigens will not be acceptable if there is greater than 80% consensus for the split.
- Serology results should be split when possible. However, parent antigens will be acceptable for serology typing only.
- Please enter results for **all five cells** in order for grading to be accurate. Otherwise, the lab may be asked to do self-grading at the end of the year.

Review, Edit and Sending Results

1. When all results have been entered for each method, click on Save and Print.
2. Review printed results to make sure all test results were entered correctly.
3. If and Edit is needed, go to Review/Edit tab to make changes.

4. After final review, click on "E-mail Files Directly" button do send files to the data manager.
5. To save the files on your hard drive, click on "Export Files for sending" and an Excel file will be saved on your computer. Some laboratories may not be able to e-mail files directly because of firewall issues at their institution. In this case, save the files to your hard drive and then e-mail them as an attached file as you would any e-mail from your institution.
6. The database manager will respond that the file has been received.

Grading Rules for HLA Typing:

Each result will be graded if there is 80% agreement. If you do not wish to be graded for a particular locus, do not enter any results. (Ex. Cw) All results will be defaulted to NT (not tested) until you actually enter a result.

Class I will be considered as an analyte. A miss of any component of the Class I locus (HLA-A,B,C) will be a miss for the sample. All results reaching 80% consensus will be graded (except Bw4,6). Bw4 and Bw6 discrepancies will be shown on the results table, but will not be graded as part of the analyte.

Class II will be considered as an analyte. A miss of any component of the Class II locus (DR, DQ) will be a miss for the sample. All results reaching 80% consensus will be graded (Except DR51,52,53). Discrepancies with DR51,52, and 53 (DRB3,4,5) will be shown on the results table, but will not be graded as part of the analyte. Results for DQA1, DOA1, and DPB1 will be graded on a separate report.

Splits must be called in the Antigen and Serology results when possible. Reporting the correct parent antigen will not be counted as a miss for Serology, but will be counted as a miss for Antigen results when consensus for the split reaches 80%. (The Antigen results are based on DNA typing). Reporting the incorrect split will always be counted as a miss for both serology and antigen results.

The AFDT Proficiency testing committee will review all results before reporting. Grading comments will be available in some cases to help explain why the result entered was counted as a miss, not counted as a miss, or not graded.

Reports will be e-mailed. Labs have one week to review and make challenges.

Results for DQA, DPA and DPB will be on a separate summary report.

Problems:

Problems with specimens or grading challenges should be brought to the attention of Arlene Skinner, skinner@seopf.org; Ph: 804-323-9890; FAX: 804-323-1300

For problems with entry of results and computer issues, please contact Marilyn Langan at molangan@sc.rr.com; Ph: 803-537-1283.

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APPENDIX E

**2013 Crossmatch/HLA Antibody Survey
Instructions**

Survey Description:

The AFDT XM/ HLA Ab Survey is designed to evaluate a laboratory's ability to detect HLA antibodies, identify antibody specificity, and determine if the antibody is of sufficient strength to result in a positive crossmatch when the sera are tested with selected cells. The HLA type of the cells will be provided to the laboratory in the accompanying paperwork. Since different centers have different criteria for assessing "compatibility", the PT program can only grade the presence or absence of antibody and cannot grade whether the level of antibody constitutes compatibility or incompatibility. However, for educational purposes, all labs will be asked to enter whether they would recommend transplantation, based on the results.

Each sendout consists of 2 whole blood samples and 5 sera. The laboratory is asked to perform 10 crossmatches (Each serum with each cell combination) using all methods used in the laboratory for which they wish to be evaluated. In addition, the laboratory will perform HLA antibody screens on each serum and, if positive, determine the antibody specificity.

There will be 2 sendouts per year for a total of 20 T cell and 20 B cell crossmatches and 10 sera for HLA antibody testing.

RESHIPMENTS WILL OCCUR <u>ONLY</u> IF SAMPLES ARE LOST OR LEAK! NO EXCEPTIONS.

<u>Software for HLA Typing and XM/PRA Proficiency Testing</u>

Application software is provided by AFDT for the HLA Typing and XM/HLA Ab surveys. A new version for 2012 and forward should have been sent to your laboratory. If you do not have this software, please contact Arlene Skinner at <u>skinner@seopf.org</u> .

When you receive the software, it will be a zipped file. Save the zipped file to your desktop. Then right click on the icon and choose "Extract All". Place the unzipped AFDT folder on the C drive. Remove the zipped folder to your Recycle bin. You can open the AFDT folder, right click on the application icon and choose "Make Shortcut" to add a shortcut to your desktop.

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The first time you use the application software, you will need to enter your laboratory name and lab code. The lab code was provided by AFDT.

Choose **Antibody/Crossmatch** to enter results. After the results have been entered, you can print the results for review and to keep for your records. Then you can directly e-mail the results back to the by Clicking "Create e-Mail File for Sending" on the HLA Typing menu page. (Some systems have firewalls that may prevent direct e-mail. In this case, select "Export files for sending" to save the files to your hard drive in the AFDT folder. You can then e-mail the file to molangan@sc.rr.com.)

For new users there are detailed application instructions sent with the software. Please review before loading and using the software the first time. For any software-related problems, please contact Marilyn Langan at <u>molangan@sc.rr.com</u>.

Results Entry:

Crossmatch
<u>The crossmatch techniques</u> that will be graded are:
CDC – T and B
AHG – T and B
Flow Cytometry- T and B
Solid Phase XM– Class I and Class II

There will be separate entries for T cell and B cell.

A laboratory can do as many of the techniques as they wish and can do either T cell or B cell or both. **However, it is important that results be reported for all the sample combinations for each technique reported**.

All results will be defaulted as NT (Not tested) unless the laboratory enters a result.

1. Open **Crossmatch/HLA Ab Survey**
2. When you start a new set of result entries, click on **Add New XM Results**
3. Fill in Serum #, Cell # and put Tech initials in Tested by: Enter Results.
4. **Add New Record**; Repeat for each serum/cell combination (10 entries)
5. **Save** Record

6. **Print/Preview XM Results**
7. Use **Review/Edit XM results** to make changes. DO NOT USE "Add New Results" when editing previously entered results as this will erase all your previous answers!
8. Press **Save and Close** to go back to Crossmatch/HLA Antibody menu.
9. Go to Enter HLA Ab Results Button

NOTE: **Weak positive crossmatches** should be recorded as Positive if significantly shifted from the Negative control. The laboratory may comment if desired. (**Example 1**: Weak Positive B cell XM correlates with presence of DSA but does not meet our center's criteria for incompatibility**. Example 2**: Weak positive T cell shift does not correlate with DSA. Auto-XM needed for interpretation of compatibility)

HLA Antibody Testing

The HLA Antibody techniques that will be graded are:
HLA Ab Screen - Solid Phase (Pos/Neg method)
Serology - CDC/AHG
Solid Phase - Phenotypic Panel - results from one or combination of panels used
Solid Phase - Single Antigen Panel- results from one or combination of panels used

Class I and Class II will be graded separately for each of the above.

HLA Ab Screen- Solid Phase

The HLA Ab screening results will be (P)os or (N)egative; Do not enter a percentage or enter a result if a screening method (Yes/No answer) was not used.
This is to be filled **out ONLY by labs using SOLID PHASE screening methods**. CDC results should be entered by choosing CDC/AHG from the Test Name dropdown menu so that it is not graded with the more sensitive solid phase techniques.

1. Choose **Enter HLA Ab Results**
2. Fill in Sample number and Tested by: (Tech initials).
3. Click on **Test Name** and choose which result you will be entering: Ex. Class I screen
4. Choose **Method** used. If a lab used more than one method, be sure to **Cntl/click** on each method that was used to obtain the results reported for this test.

5. Record result; If the screen is positive, click in box to right of screening result if you will be doing antibody specificity testing.
6. Click on **Add new record** to repeat for next test name.
7. Repeat Steps 2-6 for each serum

Antibody Specificity – Solid Phase

For each serum:

1. Click on **Add new record**. Enter Sample number and Technologist initials in Tested by:
2. Click on **Test Name** and enter which result you will be entering: CI or CII ID by Phenotypic or Single antigen panels
3. There is also a **Methods** box where the lab can indicate what method was used (ex. ELISA, Flow, Microarray, etc.) If a lab used more than one method, be sure to **Cntl/click** on each method that was used to obtain the results reported for this technique.
4. Specificities should be selected by clicking in the appropriate box. There is also a comments box available. If an antibody is found that is not on the list. (note: A80 is missing from list), you can enter it in the comment box. If results are in the borderline range, they can be listed in Comments.
5. Repeat Steps 1-4 for each technique used (Test Name) and for each serum.

NOTE: If a laboratory does not use a screening method (Pos/Neg) and goes straight to specificity panels, do not fill out screening test results. However, do make a record for each serum tested under the Specificity Test Name and enter Negative in the comments if no antibodies were detected. If a lab does Screening first and only sets up Specificity testing on the Positive samples, then they will only make records for the samples that were actually tested with the Specificity method.

CDC/AHG results – special notes

Follow the directions for HLA Antibody specificity by Solid Phase above, with the following exceptions:

1. If no HLA Antibody is detected by CDC/AHG, type the word "Negative" in Comments.

2. If Positive, enter specificities
3. If Positive, but no clear specificity, enter "no clear specificity" in Comments
4. In order for the grading to be accurate, you must enter specificity when possible, even if the serology results are not routinely reported by your laboratory. Otherwise, do not report results for this method since % positive is not graded, nor is this method graded as a screening test. You can indicate in comments that your lab does not report specificity in the clinical setting, but specificities or "no clear specificity" must be entered if the sample is positive.

Review and Edit

1. When ALL records have been completed for all testing performed, click the "**Prev/Print PRA CI & CII results**".
2. Review printed results to make sure all test results were entered and saved. Check for transcription errors. Make sure that you have printed results for all tests that you entered to ensure that no results were "written over".
3. If an Edit is needed, go to the **Review/Edit** tab to make changes.
4. After final review click on the **E-mail Files directly** button to send files. For this exchange, there will be two e-mail files, one for XM and one for HLA Ab.
5. Alternatively, the files can be saved to your hard drive by clicking the "Export Files for Sending" button. The files will be saved in the AFDT folder on the C: drive. The files can then be e-mailed to molangan@sc.rr.com.

Important Notes!

1. Each test entry (Test Name) will be a SEPARATE RECORD for each serum.
2. Choose **Add New Record** to continue with the next test or next serum. (ex. CI HLA Ab screen, CI solid phase-phenotypic panel, CI-CDC, CI-single antigen panel). If you fail to do this, you may write over previous results.
3. There can be only one set of results for each Test Name on the same serum. For instance, if you do both Luminex and Flow single antigen beads, you must enter the results from both methods together under the same Test Name (ex. Class I Single Antigen Panel).
4. Enter Pos/Neg results for solid phase testing methods under the Screening Tests. <u>DO</u> **NOT put CDC screening results with this Test category.**

5.   For Specificity Testing, choose the appropriate TEST NAME (CI or CII Phenotypic panel or Single Antigen Panel). For each TEST NAME that you choose, you must provide results for ALL POSITIVE SERA. You may use more than one method under the TEST NAME, but there should be only one set of results for each test name (combine results if more than one method was used).

6.   Some labs do not do a separate screening test, but go straight to HLA Ab identification panels to determine if antibody is present or not. In this case, the lab should make a record for all sera under the Phenotypic or Single antigen panel test results. If Negative, enter "Negative" in the Comment box. Do not enter results under the "Screening" results. Screening results are only for Solid Phase screening assays (Pos/Neg screening results)

7.   For each Test Name that you choose, you must provide results for **ALL positive sera** in order for the grading to be accurate.

ASHI Accreditation recommends that a lab does PT for each method that is used as a stand-alone test for reporting results.

Grading Rules:

Crossmatch:
- Grading will be done for all combinations that reach 80% consensus.
- A separate grade will be provided for T cells and B cells (when using CDC, AHG, and Flow), and for Class I and Class II (when using Solid Phase techniques).
- A positive result will not be counted as a miss if it is confirmed by a more sensitive technique (must meet consensus).

HLA Antibody screen: (Solid Phase Screening)
- Grading will be done for results that reach 80% consensus.
- A separate grade will be provided for Class I-HLA Ab Screen(Solid Phase) and Class II-HLA Ab Screen (Solid Phase)

HLA antibody identification will be graded as follows:
- Discrepancies will be shown for specificities that reach 80% consensus. However, since all panels do not contain some of the more rare antigens, only the following specificities will be graded:
  HLA-A1,2,3,11,23,24,25,26,29,30,31,32,33,34,68
  HLA-B7,8,13,18,27,35,38,44,45,49,51,52,55,57,60,62,65
  HLA-DR1,4,7,8,9,10,11,12,13,14,15,16,17,18; DR51,52,53

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HLA-DQ 1,2,3,4,5,6,7,8,9

- Consensus will be shown for all antibodies. Discrepant results will be indicated in the "miss" row of the Results Table.

- Discrepant results for ungraded antigen (not in list above) will be shown in the Miss row of the table, but will not be included in the grading.

- A separate grade will be provided for Class I-CDC/AHG; Class II-CDC/AHG; Class I-Solid Phase Phenotypic Panel; Class II-Solid Phase Phenotypic Panel; Class I-Solid Phase Single Antigen Panel; Class II-Solid Phase Single Antigen Panel

- Partial credit will be given for all specificities that were correctly called. For example, if there were 5 specificities that reached consensus for a particular serum and the lab called four of these specificities, they will be given 80% credit for that serum. The average of the grades for each serum will be the grade for the exchange.

- Specificities that were called by a lab but were called by less than 10% of all labs will be flagged as a possible "overcall" by the laboratory.   A 5% deduction will be taken if more than five overcalls are called for a serum.

Problems: Problems with specimens or grading challenges should be brought to the attention of Arlene Skinner at: skinner@seopf.org; Ph (804) 323-9890; FAX: 804-323-1300.

Problems with entry of results and computer issues, please contact Marilyn Langan at molangan@sc.rr.com; Ph (803) 537-1283.