PT Report: ASHI Virtual Crossmatch Educational Challenge-2

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on behalf of the PT Committee

The ASHI VIRTUAL CROSSMATCH EDUCATIONAL CHALLENGE-2 (VCEC-2) was designed in Google forms as a series of questions. Questions probed participants' method(s) of HLA antibody testing, serum treatment approaches, ability to identify donor-specific HLA antibodies (DSA) in serum samples, perform virtual crossmatching (VXM) and predict outcomes of physical flow (FXM) or complement dependent cytotoxic (CDCXM) crossmatching. Lastly, labs were asked to give a basic risk assessment based on the VXM for organ specific situations that their lab would encounter.

The VCEC-2 consisted of five ASHI 2018 AC-2 sera (AC-505 -509) tested by participating laboratories and three AC-2 donor cells (AC-124, AC-126, AC-127). This was decreased from the original six donor cells to shorten the length of the challenge and focus on interesting VXM combinations. Donor HLA typing was performed using Real Time PCR single antigen bead resolution trays and provided to the participants in the survey. In total, eighty-three AC-2 participants signed up for the VCEC-2 and sixty-three AC-2 subscribers participated in the VCEC-2 survey which generated a response rate of 76 %. As shown in Table 1, the participants were divided into three groups, based on the AC-2 group status, to ensure that they were blinded to the physical XM results. Of note, AC-2 survey donor sample AC-127 did not contain significant numbers of cells for physical CDC XM testing, and therefore no physical CDCXM data was available for comparison with the VXM results.

Table 1: Virtual XM donor assignments for AC-2 participants who signed up and responded to VCEC-2 with their corresponding AC-2 physical XM donor pair.

AC-2 Participants who signed up for VCEC-2	VCEC-2 Responders	Physical XM Donors	Virtual XM Donors
Group A (N=23)	Group C (N=20)	AC-123/AC-124	AC-126
Group B (N=26)	Group A (N=24)	AC-125/AC-126	AC-127
Group C (N=26)	Group B (N=19)	AC-127/AC-128	AC-124
Group D (N=8)		None	

Participants were first asked about the type of serum treatment used by their laboratory in the ASHI 2018 AC-2 survey. Overall, the majority of laboratories (51.0 %) used EDTA to treat sera followed by 25.4 % of labs who did not perform any serum treatment. Other treatments included DTT (12.7 %), Heat Inactivation (4.8 %), Heat Inactivation and FCS (1.6 %), DTT and FCS (1.6 %) and Absorb-out beads (1.6 %). The second question asked the participants to identify the vendor of the single antigen bead (SAB) assay their laboratory used to test the 2018 AC-2 survey sera. In all groups, a majority of labs reported the LABScreen SAB assay (OneLambda) alone for serum testing. Furthermore, 4.2 % of participants in Group A and 5.6 % in Group B used Lifecodes LSA SAB (Immucor) only while 5.9 % of Group C used both SAB vendors. (Figure 1).

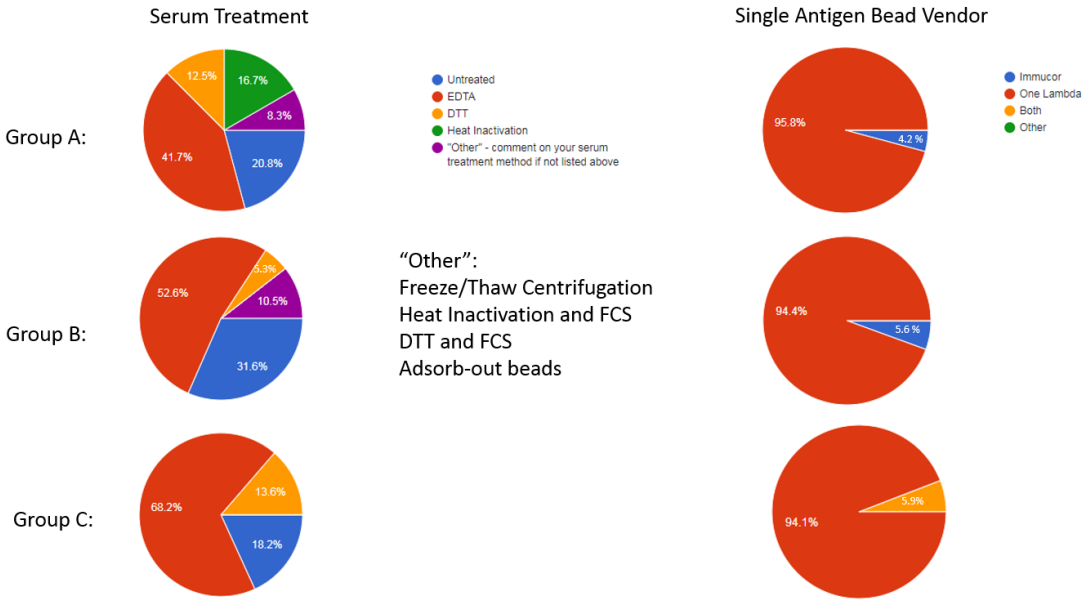


Figure 1: VCEC-2 group responses to type of serum treatment performed and chosen single antigen bead vendor.

Next, the participants were asked to identify any DSA they detected for each serum/donor cell combination specified with MFI values deemed "Positive" based on their established MFI cutoff in antibody identification testing. They were also asked to indicate if VXM was positive or negative and predict the outcome of the FXM and CDCXM. Finally, we asked the participants to provide a risk assessment for organ specific situations including Kidney, Heart, Lung, Pancreas, Kidney/Pancreas, Kidney/Liver that their laboratory would encounter given the DSA they detected.

For this VXM educational challenge we provided several assumptions including: there is no matching between HLA typings of donor and recipient pairs, each recipient has had no significant clinical events to consider, the participants have tested current serum samples and the patient's anti-HLA antibody testing history is consistent with the current test results.

All the VCEC-2 data collected were linked to Google sheets and exported to Microsoft Excel and GraphPad Prism for data analysis. Figure 2 represents AC-2 Group A/VCEC-2 Group C results for the AC-124 donor cell vs AC-505 - 509 sera including all serum treatments and single antigen bead vendors. Comparison of T cell and B cell physical versus virtual FXM and CDCXM were performed. Overall, VXM results were similar to the physical XM results (Figure 2A). There was a high agreement between VXM predicting a FCXM and the physical FCXM result across all VCEC-2 groups. Comparison of AC-126 donor cell vs AC-505 serum demonstrated a lower VXM positive prediction (74 %) compared to a 100 % physical FCXM result. There were low level anti-HLA antibodies detected against A*01:01 and DRB3*01:01. Additionally, DRB1*03:01 and DQ7 had more variation including low MFIs which

potentially influenced more participants to predict a negative VXM. In all VCEC-2 groups there was a tendency for the VXM participants to overestimate physical CDCXM positivity (Figure 2A). Within the risk assessment per organ, higher MFI levels of DSA, above 5000 MFI, (Figure 2B) led to high risk responses (Figure 2C), whereas low level DSA or DSA to lower expressing HLA-C antigens lead to differences in risk stratification (Figure 2B and 2C). In cases where a majority of organs considered the VXM response as high risk, they were more likely to be considered moderate or low risk for Kidney/Liver.

Complete access to all of the graphs and data tables can be found **here** and on the PT page of the ASHI website. A more in-depth analysis will follow in a later PT Committee publication.

Figure 2 A, B, C

Figure 2A. Comparison of percent positive T cell and B cell FCXM Physical vs Virtual and T cell and B cell CDC-XM Physical vs Virtual.

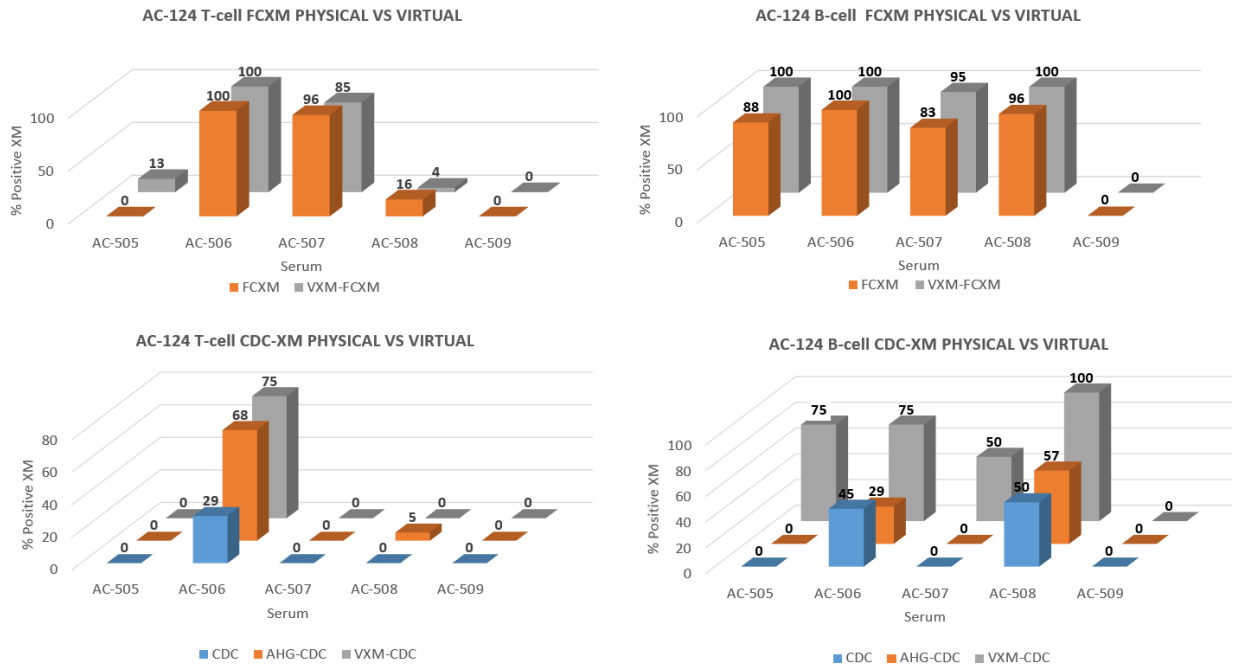
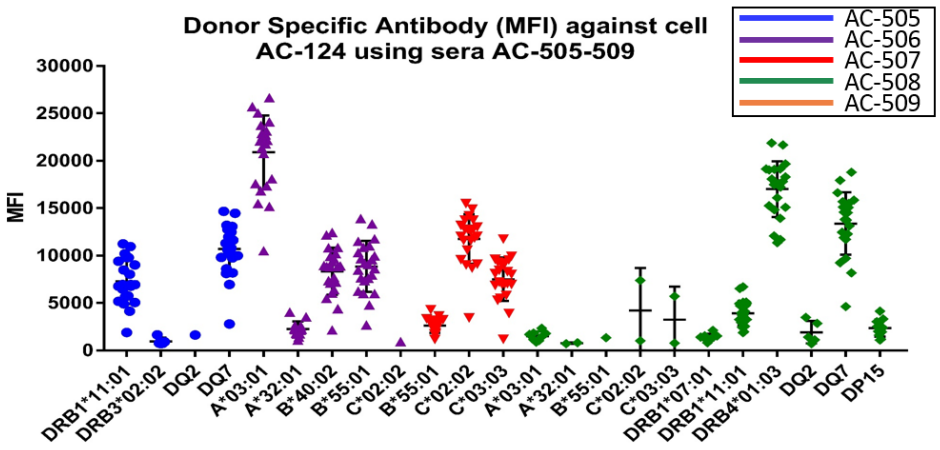


Figure 2B. Donor specific antibody (MFI) against cell AC-124 using sera AC-505-509.



# of values	22	5	1	22	23	13	22	23	1	19	22	22	8	2	1	2	2	8	22	22	5	22	11
Mean	7283	954.8	1613	10682	20895	2258	8343	8853	923	2617	11751	7501	1508	762.8	1330	4197	3236	1400	3929	17003	1911	13370	2354
SD	2393	396.1	0	2682	3896	812.7	2484	2687	0	763.6	2626	2286	492.5	68.99	0	4511	3485	368.8	1306	2935	1196	3288	920.9
CV%	32.86%	41.49%	0.00%	25.11%	18.65%	35.99%	29.77%	30.35%	0.00%	29.18%	22.35%	30.48%	32.67%	9.04%	0.00%	107.49%	107.72%	26.35%	33.22%	17.26%	62.59%	24.59%	39.12%

Figure 2C. Virtual Crossmatch Risk Assessment Response Separated by Sera and Organ Case.