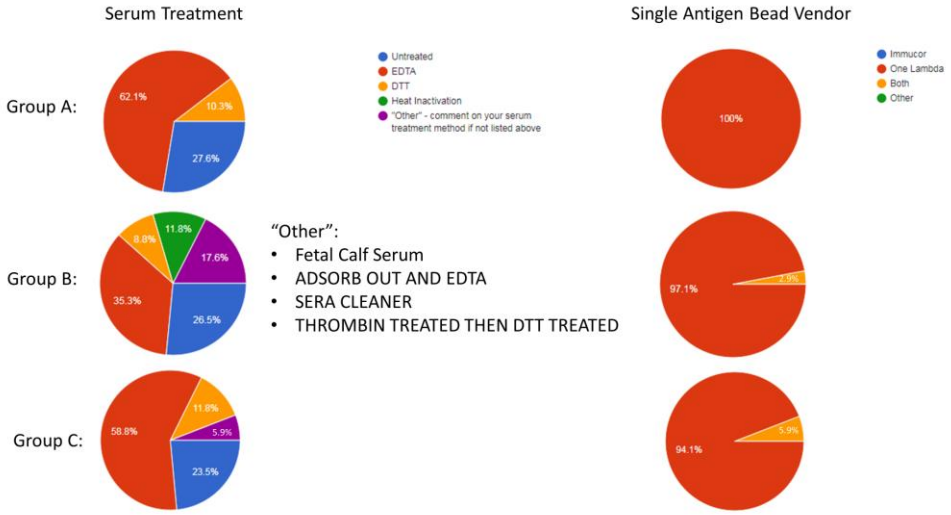
ASHI VIRTUAL CROSSMATCH EDUCATIONAL CHALLENGE

The ASHI VIRTUAL CROSSMATCH EDUCATIONAL CHALLENGE (VCEC) was designed in Google forms as a series of questions. Questions probed participants' method(s) of HLA antibody testing, abilities 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 consisted of five ASHI 2018 AC-1 sera (AC-500 -504) tested by participating laboratories and six AC-1 donor cells (AC-117 – 122). Donor HLA typing was performed using Real Time PCR single antigen bead resolution trays and provided to the participants in the survey. In total, there were eighty four AC-1 subscribers who participated in the VCEC survey. As shown in Table 1, the participants were divided into three groups, based on the AC-1 group status, to ensure that they were blinded to the physical XM results. Of note, AC-1 survey donor sample AC-121 did not contain significant numbers of B cells for physical XM testing, and therefore no data was available for comparison with the VXM results.

AC-1 Group	VCEC Group	Physical XM Donors	Virtual XM Donors
Group A	Group C	AC-117/AC-118	AC-121/AC-122
Group B	Group A	AC-119/AC-120	AC-117/AC-118
Group C	Group B	AC-121/AC-122	AC-119/AC-120
Group D	Group B	None	AC-119/AC-120

Participants were first asked about the type of serum treatment used by their laboratory in the ASHI 2018 AC-1 survey. Overall, the majority of laboratories (52.0 %) used EDTA to treat sera followed by 25.7 % of labs who did not perform any serum treatment. Other treatments included DTT (9.7 %), Heat Inactivation (7.3 %), Fetal Calf Serum (2.4 %), Fetal Calf Serum and DTT (1.2 %), AbsorbOUT with EDTA (1.2 %), DTBA (1.2 %), Sera Cleaner (1.2 %), and Thrombin-treated then DTT-treated (2.4 %). 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-1 survey sera. In all groups, 100% of labs used the LABScreen SAB assay (OneLambda) for serum testing. Furthermore, 2.9% of participants in Group B and 5.9% in Group C used Lifecodes LSA SAB (Immucor) in addition to the LABSccreen SAB assay (Figure 1).



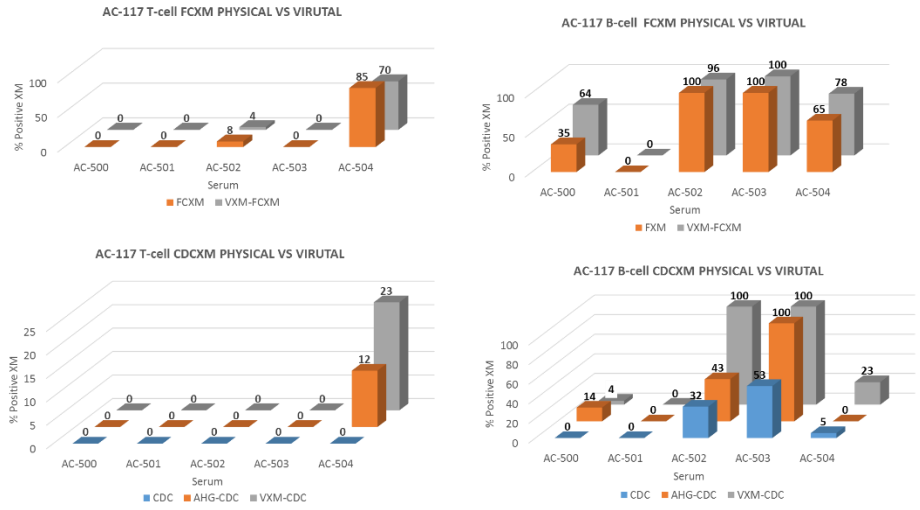
Next, the participants were asked to identify any DSA they detected for each serum/donor cell combination specified with MFI values deemed "Positive" in antibody identification testing. They were also asked to indicate if VXM was positive or negative and predict the outcome of the FXM and CDCXM. Finally, 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.

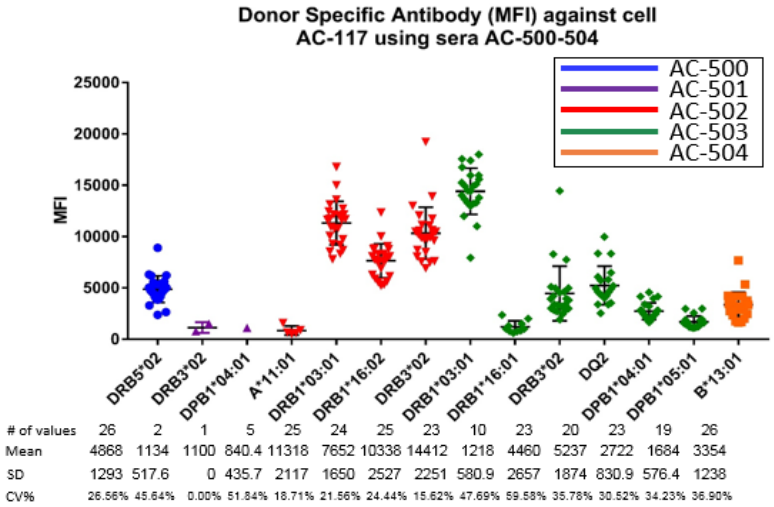
For this VXM educational challenge we provided several assumptions including: there is no matching between HLA typings of donor and recipient pairs, each recipient has had no significant clinical events to consider, the participants have tested current serum samples and the patient's anti-HLA antibody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

All the VCEC data collected was linked to Google sheets and exported to Microsoft Excel and GraphPad Prism for data analysis. Figure 2 represents AC-1 Group A/VCEC Group C results for the AC-117 donor cell vs AC-500 -504 sera. Comparison of T cell and B cell physical versus virtual FXM and CDCXM was performed. Overall, VXM results were similar to the physical XM results (Figure 2A). However, there was a tendency for the VXM participants to overestimate physical FXM and/or CDCXM positivity (Figure 2A). Within the risk assessment per organ, higher MFI levels of DSA or number of DSA (Figure 2B) led to high risk responses (Figure 2C), whereas low level DSA or single DSA 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.

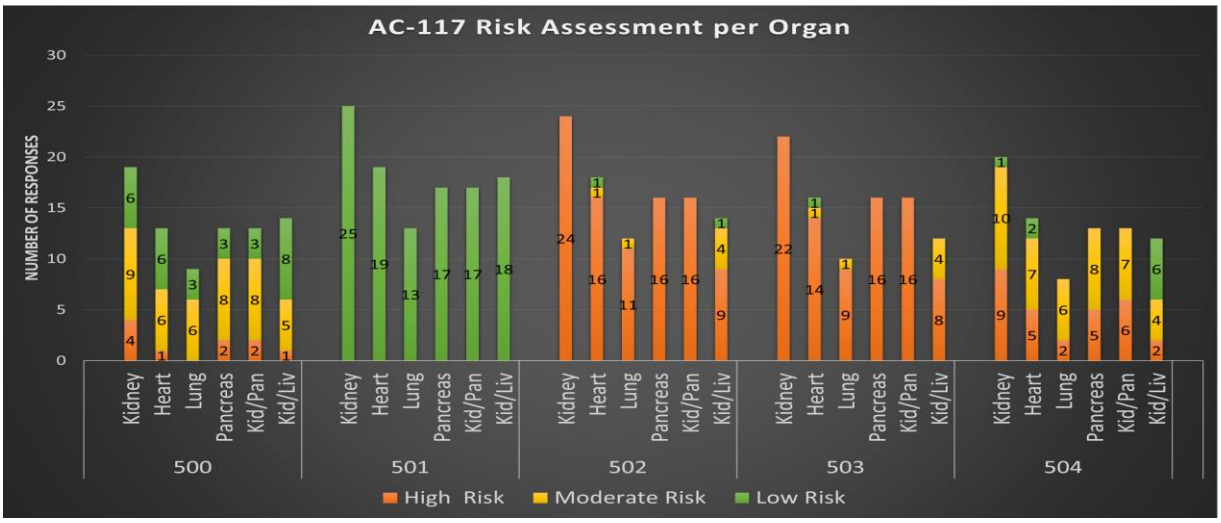
Complete access to all of the graphs and data tables can be found on the ASHI website's PT page. This article is also included in the 4th quarter edition of the 2018 Quarterly newsletter. (to be released during the 1st quarter of 2019)

Figure 2 A, B, C





	DRB5*02	DRB3*02	DPB1*04:01	A*11:01	DRB1*03:01	DRB1*16:02	DRB3*02	DRB1*03:01	DRB1*16:01	DRB3*02	DQ2	DPB1*04:01	DPB1*05:01	B*13:01
# of values	26	2	1	5	25	24	25	23	10	23	20	23	19	26
Mean	4868	1134	1100	840.4	11318	7652	10338	14412	1218	4460	5237	2722	1684	3354
SD	1293	517.6	0	435.7	2117	1650	2527	2251	580.9	2657	1874	830.9	576.4	1238
CV%	26.56%	45.64%	0.00%	51.84%	18.71%	21.56%	24.44%	15.62%	47.69%	59.58%	35.78%	30.52%	34.23%	36.90%


AC-117 Risk Assessment per Organ
NUMBER OF RESPONSES
30
25
20
15
10
5
0
Kidney
Heart
Lung
Pancreas
Kid/Pan
Kid/Liv
500
501
502
503
504
High Risk
Moderate Risk
Low Risk