ASHI 2020 Virtual Crossmatch Educational Challenge-1
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The 2020 ASHI VIRTUAL CROSSMATCH-1 (VXM-1) EDUCATIONAL CHALLENGE was again designed in Google forms as a questionnaire. Participants in this challenge were asked to perform a virtual crossmatch assessment using HLA antibody data obtained from the ASHI AC-1 2020 survey. The virtual crossmatch assessment included a prediction of the outcome of physical flow cytometric (FCXM) or complement dependent cytotoxic (CDCXM) crossmatches. The questions also probed participants’ use of one versus two single antigen panels for HLA antibody testing, serum treatment and determination of donor-specific HLA antibodies (DSA). Finally, the participating laboratories were asked to use the data from the VXM to provide a risk assessment in the case of kidney, heart, pancreas, kidney/heart and kidney/liver transplantation.

The VXM-1 consisted of five sera tested in the ASHI 2020 AC-1 survey (AC-520 through AC-524) and three AC-1 donor cells (AC-141, AC-143, AC-146). The HLA typing for donor cells (Table 2, 3 and 4) was performed at intermediate resolution by SSOP and the results were provided in the survey. Of 147 participants from the 2020 AC-1 survey, 54 (36.7 %) subscribers participated in the VXM-1 survey. By comparison, there were 65 and 49 participants in the 2019 VXM-1 and -2 challenges, respectively. As shown in Table 1, the participants were divided into three groups. Each group was assigned a donor cell that is different from the donor cell assigned for the ASHI 2020 AC-1 survey and, therefore, participants were blinded to the physical crossmatch results. The VXM assessments were compared to the physical CDCXM and FCXM results obtained from the 2020 AC-1 survey. All of the 2020 VXM-1 data collected were linked to Google sheets and exported to Microsoft Excel and GraphPad Prism for data analysis.

Table 1. Number of 2020 VXM-1 Participants and cell assignments: Each group was assigned a donor cell that was not tested by this group for their cell-based crossmatch in the AC-1 survey.

<table>
<thead>
<tr>
<th># of Responses per group</th>
<th>Donor cell assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (N=13)</td>
<td>AC-141</td>
</tr>
<tr>
<td>Group B (N=17)</td>
<td>AC-143</td>
</tr>
<tr>
<td>Group C (N=24)</td>
<td>AC-146</td>
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Serum treatment for antibody assessment

Participants were asked to report their laboratories standard serum treatments used for antibody testing. The majority (81%) of laboratories who participated in the 2020 VXM-1 reported use of a single serum treatment; 47% used EDTA, 21% used DTT, 8% used heat inactivation and 7% used other methods (not specified by the laboratory). Additionally, 18% of laboratories did not use any serum treatment (untreated serum) prior to antibody testing. The distribution of serum treatment per group is detailed in Figure 1.
Single versus Multiple Assays used for Antibody Assessment
On average, 93% of the participants reported using only one single antigen panel which is distributed more heavily towards one particular vendor, while 7% of the participants used two different single antigen panels to report antibody. The distribution of assay per group is shown in Figure 2.

Donor Specific Antibody (DSA) Detected, Crossmatch Comparison and Risk assessment
The participants were asked to identify any DSA detected for each serum/donor cell combination with MFI values deemed "Positive" based on their established MFI cutoff. DSA and MFI reported are listed for each group in Figures 3 (Group A), 6 (Group B) and 9 (Group C). All graphs depict box plots with normalized MFI values and ranges. “X” are the mean and the lines are the median values. Antigens for which at least one laboratory reported DSA are represented in box plots. The VXM were compared to the results of the physical crossmatches in Figures 4 (Group A), 7 (Group B) and 10 (Group C). Finally, risk assessments for each solid organ group and based on virtual crossmatch results are listed per group in Figures 5 (Group A), 8 (Group B) and 11 (Group C).

- Group A Results:
Table 2. HLA typing for donor cells AC-141:
Figure 3: DSA reported per serum per group A: Overall MFI raw values were between 1 and 20% higher than normalized values. There were no significant differences regarding MFI values based on different sera treatment including the no treatment group. Donor specific antibody against AC-141 was detected on sera AC-520 (Fig. 3A), AC-522 (Fig. 3B), AC-523 (Fig. 3C) and AC-524 (Fig. 3D). For serum AC-522 and AC-523, 9 labs and 8 labs, respectively, acknowledged the lack of allele C*15:04 on the panel beads they used. From these labs, only 6 and 5 labs, respectively, reported MFI using C*15:02 as surrogate allele.

Figure 4: T and B-cells XM results with cell AC-141. Overall, participants were more likely to predict positive CDC virtual crossmatches for T and B cell CDC crossmatches, which was not confirmed with the physical crossmatches. The virtual flow crossmatch predictions were in agreement with the physical crossmatch results for all sera tested against this cell. In particular for cell AC-141 versus serum AC-523, 100% of the labs predicted a positive T cell and 86% a positive B cell CDC XM due to presence of B35 and B51 DSA at MFI ranging between 8,000-12,000 MFI each, while only 17% and 14% of the physical CDC XM were reported positive for T and B cells, respectively.
Figure 5: Risk Assessment with cell AC-141:

For all sera, variability in risk assessment was mostly observed for kidney/liver transplantation.
Group A data summary:

For all sera combination with AC-141, except for serum AC-522, there was a good correlation between, DSA, physical crossmatches and virtual crossmatches. Interestingly, the combination AC-141 with serum AC-522 (with DSA A*02:01, ranging from 5,500 to 1,500 MFI and C*15:04, ranging from 1,800 to 1,100 MFI using C*15:02 as surrogate; Figure 3B) was reported as virtual T and B-cell FXM positive by 77% of the participants, but only 44% and 20% of the labs reported it positive by physical T and B-cell FCXM, respectively (Figure 4). In concordance, this combination showed greatest variability in risk assessment across all organs between all participants (Figure 5).

- Group B Results:

Table 3. HLA typing for donor cells AC-143:

<table>
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<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>DRB1</th>
<th>DRB3/4/5</th>
<th>DQA1</th>
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<td>01:01</td>
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<tr>
<td>-</td>
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<td>07:01</td>
<td>NP</td>
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<td>05:01</td>
<td>-</td>
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Figure 6: DSA reported per serum per group B: Raw MFI values were between 1 and 16 % higher than normalized values and there were no significant differences between sera different treatments including the no treatment group. The two labs using Vendor 2 consistently reported the lowest DSA values. No antibody directed against cell AC-143 antigens was detected in sera AC-520 and AC-521. Donor specific antibody against AC-143 was detected in sera AC-522 (Fig. 6A), AC-523 (Fig. 6B) and AC-524 (Fig. 6C).
Figure 7: T and B-cells XM results with cell AC-143. For AC-520 with cell AC-143 all the labs but one reported all virtual XMs (CDCXM and FCXM) as negative, in concordance with the lack of any HLA DSA. A single lab reported positive VCDC XM and VFXM for B-cells only. In contrast, the physical B-cell FCXM that was reported positive by 33% (13) of the labs, most probably due to weak DSA against DR7.
Figure 8: Risk Assessment with cell AC-143.
Group B Results:

Virtual crossmatch by CDC was reported positive more frequently than correspondent physical crossmatches. Virtual and physical FCXM were in agreement. Risk assessment between AC-143 and serum AC-522 was variable between labs and also between organs. This combination has DSA B*40:01(60) reported between 800 and 7,700 MFI and most of the labs reported physical and virtual FCXM positive (Figure 7).

- Group C Results:

Table 4. HLA typing for donor cells AC-146

<table>
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<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>DRB1</th>
<th>DRB3/4/5</th>
<th>DQA1</th>
<th>DQB1</th>
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</table>

Figure 9: DSA reported per serum per group C: In general, MFI raw values were between 1 and 20% higher than normalized values. There were no significant differences regarding MFI values based on different sera treatments and including the untreated group. No antibody directed against cell HLA-AC-146 antigens was detected in serum AC-521. Donor specific antibody against AC-146 was detected in sera AC-520 (Fig. 9A), AC-522 (Fig. 9B), AC-523 (Fig. 9C) and AC-524 (Fig. 9D). One lab did not specify MFI values for the reported DSA. For AC-522, two labs using Vendor 2 kit did not report A*02:01 as DSA. Since AC-146 cell is typed as C*07:01, which is not present on bead panel from Vendor 1, some labs using this vendor reported MFI for bead C*07:02 as surrogate for sera AC-522 (5 labs) and AC-523 (15 labs).
Figure 10: T and B-cells XM results with cell AC-146. One lab reported T-cell VXM with serum AC-520 as positive, despite no HLA class I DSA present.
Group C Results:

Overall, the proportion of labs reporting virtual CDC crossmatches as positive was higher than the physical crossmatches reporting as positive. Physical and virtual flow crossmatches were in agreement. Risk assessment for the combination AC-146 with sera AC-520 and AC-522 were very variable between participants and across different organs. In both cases the DSA present was at moderate levels (2,000 to 8,000 MFI) (Figure 9B).