ASHI 2020 Virtual Crossmatch Educational Challenge-2

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The 2020 ASHI VIRTUAL CROSSMATCH-2 (VXM-2) 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-2 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-2 consisted of five sera tested in the ASHI 2020 AC-2 survey (AC-525 through AC-529) and three AC-2 donor cells (AC-147, AC-149, AC-152). 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 118 participants from the 2020 AC-2 survey, 51 (43%) subscribers participated in the VXM-2 survey. By comparison, there were 65 participants in the 2019 VXM-1, 49 in the 2019 VXM-2 and 54 in the 2020 VXM-1 challenges. 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 cells assigned for the ASHI 2020 AC-2 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-2 survey. All of the 2020 VXM-2 data collected were linked to Google sheets and exported to Microsoft Excel and GraphPad Prism for data analysis.

Table 1. Number of 2020 VXM-2 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-2 survey.

<table>
<thead>
<tr>
<th># of Responses per group</th>
<th>Donor cell assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (N=14)</td>
<td>AC-149</td>
</tr>
<tr>
<td>Group B (N=14)</td>
<td>AC-152</td>
</tr>
<tr>
<td>Group C (N=23)</td>
<td>AC-147</td>
</tr>
</tbody>
</table>

Serum treatment for antibody assessment

Participants were asked to report their laboratory standard serum treatments used for antibody testing. The majority (78%) of laboratories who participated in the 2020 VXM-2 reported use of a single serum treatment; 49% used EDTA, 18% used DTT, 10% used heat inactivation and 1% used other methods (not specified by the laboratory). Additionally, 22% 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.

**Figure 1: Serum treatment per group.**

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**Single versus Multiple Assays used for Antibody Assessment**

On average, 97% of the participants reported using only one single antigen panel which is distributed more heavily towards one particular vendor, while 3% of the participants used the two different single antigen panels to report antibody. The distribution of assay per group is shown in Figure 2.

**Figure 2: Vendors used per group:**

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**Donor Specific Antibody (DSA) Detected, Crossmatch Comparison and Risk assessment**

The participants were asked to identify any DSA detected for each serum/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” represents the mean and the lines show the median values. Antigens for which at least one laboratory reported DSA are represented in box plots. Foot tables show Mean and SD for normalized MFI, when at least 3 labs reported the DSA. 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-149:

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>DRB1</th>
<th>DRB3/4/5</th>
<th>DQA1</th>
<th>DQB1</th>
<th>DPA1</th>
<th>DPB1</th>
</tr>
</thead>
<tbody>
<tr>
<td>01:01</td>
<td>08:01</td>
<td>07:01</td>
<td>03:01</td>
<td>3*01:01 (52)</td>
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<td>02:01</td>
<td>01:03</td>
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<td></td>
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<td>02:01</td>
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<td>12:03</td>
<td>13:01</td>
<td>-</td>
<td>05:01</td>
<td>06:03</td>
<td>-</td>
<td>04:02</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 3: DSA reported per serum per group A:** Overall MFI raw values (reported on the Y axis of each graph) were between 1 and 12% higher than normalized values. Donor specific antibody against cell AC-149 was detected in sera AC-525 (Fig. 3A), AC-526 (Fig. 3B), AC-527 (Fig. 3C) and AC-529 (Fig. 3D). For serum AC-526, one lab using untreated serum reported the highest MFIs per each DSA. For serum AC-529, only one lab reported antibodies against B*08:01.
**Figure 4: T and B-cells XM results with cell AC-149.** Overall, participants were more likely to predict positive CDC virtual crossmatches for T and B cells that were not confirmed with the physical crossmatches. The virtual flow crossmatch (VFXM) predictions were in agreement with the physical crossmatch results for all sera tested against AC-149 cells.

![Figure 4: AC-149 T XM](image)

![Figure 4: AC-149 B XM](image)

**Figure 5: Risk Assessment with cell AC-149:**

For all sera, variability in risk assessment was mostly observed for kidney/liver transplantation.

![Figure 5: AC-149](image)

**Group A data summary:**

For all sera combination with AC-149, there was a good correlation between DSA, physical crossmatches and virtual crossmatches. For serum AC-529, 7% of labs reported positive physical FXM despite no consensus for class I DSA present. All labs reported VFXM T-cell negative, including the few labs reporting low levels of class I DSA.

- **Group B Results:**
Table 3. HLA typing for donor cells AC-152:

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>DRB1</th>
<th>DRB3/4/5</th>
<th>DQA1</th>
<th>DQB1</th>
<th>DPA1</th>
<th>DPB1</th>
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<tbody>
<tr>
<td>03:01</td>
<td>15:01</td>
<td>01:02</td>
<td>01:01</td>
<td>4*01:01P</td>
<td>01:01P</td>
<td>02:02</td>
<td>02:01</td>
<td>05:01</td>
<td></td>
</tr>
<tr>
<td>24:02</td>
<td>40:02(61)</td>
<td>02:02</td>
<td>07:01</td>
<td>NP</td>
<td>02:01</td>
<td>05:01</td>
<td>02:02</td>
<td>14:01</td>
<td></td>
</tr>
</tbody>
</table>

Figure 6: DSA reported per serum per group B: Raw MFI values were between 1 and 22% higher than normalized values and there were no significant differences between the different serum treatments including the no treatment group. Two labs reported summed MFI for some of the DSA, and they have been excluded in this analysis. No antibody directed against cell AC-152 antigens was detected in sera AC-526 and AC-528. DSA against AC-152 was detected in sera AC-525 (Fig. 6A), AC-527 (Fig. 6B) and AC-529 (Fig. 6C). For AC-529, only one lab reported low levels of DSA against B*40:02.

Figure 7: T and B-cells XM results with cell AC-152.

No laboratory reported physical CDC XM results for either T or B cells (AC-2 survey), therefore VCDC XM cannot be evaluated. Physical and virtual FXM were in correlation.
Figure 8: Risk Assessment with cell AC-152.

Variability in risk assessment was mostly observed for kidney/liver transplantation. There was significant variation within the pancreas transplant group with serum/cell combination AC-529 versus AC-152 (the highest DSA was DRB4*01:01 mean 17847 MFI)

Group B data summary:

Overall reported XM results were in agreement with the presence of DSA. For AC-529, there was no consensus for T-cell FXM physical or virtual results. Most of the lab reported VFXM-T cell negative, and only of the few labs that detected presence of class I DSA, reported positive T-cell VFXM. This variability was also reflected in the risk assessment with this serum.

- Group C Results:
Table 4. HLA typing for donor cells AC-147

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>DRB1</th>
<th>DRB3/4/5</th>
<th>DQA1</th>
<th>DQB1</th>
<th>DPA1</th>
<th>DPB1</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>26:01</td>
<td>35:01</td>
<td>03:04 (10)</td>
<td>01:01</td>
<td>4*01:01 (53)</td>
<td>01:01</td>
<td>02:02</td>
<td>01:03</td>
<td>04:01</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>40:01 (60)</td>
<td>04:01</td>
<td>07:01</td>
<td>-</td>
<td>02:01</td>
<td>05:01</td>
<td>-</td>
<td>04:02</td>
</tr>
</tbody>
</table>

Figure 9: DSA reported per serum per group C: In general, MFI raw values were between 1 and 22% higher than normalized values. There were no significant differences regarding MFI values based on different sera treatments and including the untreated group. DSA against cell AC-147 was detected in sera AC-525 (Fig. 9A), AC-526 (Fig. 9B), AC-527 (Fig. 9C) and AC-529 (Fig. 9D). For serum AC-526, only one lab reported DSA (A*26:01) present and another lab commented on the detection of antibodies against DQA1*02:01, but not in conjunction with DQB1*02:02. Since donor is typed as DQA1*02:01-DQB1*02:02, this antibody was not considered DSA in this analysis and was not depicted in Fig 9B.

Figure 10: T and B-cells XM results with cell AC-147. Overall, there was a high correlation between physical results and virtual CDC-XM and FXM predictions. For serum AC-529, all labs predicted VCDC B-
cell positive based on the presence of high levels of antibodies against donor HLA antigens, but only 26% of the labs performing CDC-B cell XM reported positive results.

**Figure 11: Risk Assessment with cell AC-147**

Variability in risk assessment was mostly observed for kidney/liver transplantation.

**Group C data summary:**

Overall, the proportion of labs reporting virtual CDC crossmatches as positive was higher than the physical crossmatches. Physical and virtual flow crossmatches were in agreement.
Also, for serum AC-529, one lab predicted VCDC-T cell positive despite class I DSA being only detected by few labs and only at low levels.

Across all sera, the highest risk assessment variability was for kidney/liver patients. In addition, the serum with less consensus in risk assessment was AC-527.

The ASHI PT committee would like to thank the laboratories that participated in the ASHI virtual crossmatch challenges over the last three years. The committee has gathered a significant amount of data which has been shared in ASHI publications and reports. As we look to move forward in 2021, the PT Committee must determine whether the ASHI VXM challenge will be offered in 2021 to ASHI PT subscribers or if the challenge has served its purpose and should be discontinued.

Earlier feedback from participants included suggestions for enhancing the ASHI VXM experience. If the decision is made to continue with the VXM challenge, the PT committee will be incorporating participants' suggestions to streamline and improve the result entry process. A few of the suggestions that will be explored are decreasing the number of questions, focusing on interesting cell versus serum combinations, and giving participants access to result data should they wish to perform their own analysis.

If you would like to provide any additional feedback on whether the VXM challenge should continue to be offered or suggestions for improvements, these suggestions will also be taken into consideration if the VXM challenge is offered in the future. Comments and suggestions about the VXM challenge can be emailed to info@ashi-hla.org. Your continued feedback will help guide the PT Committee in determining whether this challenge has reached the end of its usefulness or whether it should continue to be a part of the ASHI PT educational offerings.