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## Authors
Locke, Arlene
Hickey, Michelle
Reed, Elaine
et al.

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# Virtual and Reality: An Analysis of the UCLA Virtual Crossmatch Exchanges

Arlene F. Locke, BS,[1] Michelle Hickey, PhD,[1] Nicole M. Valenzuela, PhD,[1] Carrie Butler, PhD,[1] Rebecca Sosa, PhD,[1] Ying Zheng, MPH, MS,[1] David Gjertson, PhD,[1] Elaine F. Reed, PhD,[1] and Qiuheng Zhang, PhD[1]

**Abstract.** The "virtual" crossmatch (VXM) has become a critical tool to predict the compatibility between an organ donor and a potential recipient. Yet, nonstandardized laboratory practice can lead to variability in VXM interpretation. Therefore, UCLA's VXM Exchange survey was designed to understand factors that influence the variability of VXM prediction in the presence of HLA donor-specific antibody (DSA). Thirty-six donor blood samples and 72 HLA reference sera were sent to 35 participating laboratories to perform HLA antibody testing, flow crossmatch (FXM), and VXM from 2014 to 2019, consisting of 144 T/B-cell FXM pairs and 112 T/B-cell VXM pairs. In the FXM survey, 86% T-cell FXM and 84% B-cell FXM achieved >80% concordance among laboratories. In the VXM survey, 81% T-cell VXM and 80% VXM achieved >80% concordance. The concordance between FXM and VXM was 79% for T cell and 87% for B cell. The consensus between VXM and FXM was high with strong DSA. However, significant variability was observed in sera with (1) very high titer antibodies that exit prozone effect; (2) weak-to-moderate DSA, particularly in the presence of multiple weak DSAs; and (3) DSA against lowly expressed antigens. With the increasing use the VXM, standardization and continuous learning via exchange surveys will provide better understanding and quality controls for VXM to improve accuracy across all centers.

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## INTRODUCTION

For transplant candidates, the presence of preformed anti-HLA antibodies against the donor is a major barrier for successful renal,[1] heart,[2,3] lung,[4] liver,[5] intestine,[6] and hematopoietic stem cell transplantation.[7] Laboratory testing for organ donor and recipient compatibility has undergone several major technical advances since the development of the complement dependent cytotoxicity assay over 50 y ago.[8] An array of histocompatibility and immunogenetics laboratory tests has evolved to identify the strength and specificity of HLA donor-specific antibody (DSA) at the time of a donor offer to prevent organ rejection.

Decision-making based on accurate laboratory data is crucial at the time of the donor offer as organs are allocated nationally and regionally from both the UNOS Kidney Allocation System for deceased donor organs and through registries for living donation. Before acceptance, the patient's history of histocompatibility data is reviewed, and many laboratories perform virtual crossmatches (VXMs) to determine the presence or absence of preformed DSA and to predict the result of a flow cytometry crossmatch (FXM) and correlation to posttransplant risk of rejection. The accuracy of the VXM is crucial for safe organ transplant, particularly for sensitized patients receiving organs recovered from donors at geographically distant sites from the patient's transplant center. It is estimated that ~20% of deceased donor kidneys are transplanted into a recipient other than the primary intended recipient because of an unexpected or unacceptably positive physical FXM, prolonging the cold ischemia time for the organ.[9] The use of the VXM reduces cold ischemia time, allows for the allocation of donor organs nationally, and reduces wait time and waitlist mortality in kidney transplant recipients.[10-13]

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[1] *UCLA Immunogenetics Center, Department of Pathology and Laboratory Medicine, UCLA Health, Los Angeles, CA.*

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*Correspondence: Qiuheng Zhang, PhD, UCLA Immunogenetics Center, Department of Pathology and Laboratory Medicine, David Geffen School of Medicine at UCLA, Rehab Building, Rm 1520, Los Angeles, CA 90095. (jqzhang@mednet.ucla.edu).*



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Laboratories participate in proficiency testing and surveys to assess performance among peers according to CMS regulations. However, thus far, proficiency testing is limited to each test and analyte but does not assess performance of the entire array of tests or personnel competency used in the decision-making before transplant donor acceptance. To address this gap, we developed the UCLA VXM Exchange—a 2-phase challenge that assesses laboratory consensus in HLA antibody detection and VXM and FXM reporting. The accuracy of VXM in predicting negative FXM in patients without HLA DSA is high.[14] However, VXM concordance with FXM can be lower in the presence of DSA for several reasons.[15] Technical limitations in each particular assay such as interfering substances in patient serum (IgM or complement),[16] nonspecific antibody binding,[17] antigen expression on donor cells or antigen "load" on the solid-phase bead array, and antibody strength and specificity may confound the VXM. An advantage of this exchange is the added opportunity to learn about instances of discordance between the VXM and FXM that may be due to these limitations.

## MATERIALS AND METHODS

### Study Description and Data Collection

The UCLA VXM Exchange is the first program designed to provide laboratories with the opportunity to compare VXM with an actual FXM in 3 unique surveys per year. The survey is divided into 2 phases (Figure 1). In Phase I, participating laboratories are sent 2 sera for HLA class I and class II antibody testing by single antigen bead test (SAB). The laboratories will then perform 8 VXM against 4 virtual donors with complete HLA typing (HLA-A, B, C, DRB1/3/4/5, DQA1, DQB1, DPA1, and DPB1). Each VXM challenge is given a fictional clinical vignette including if the patient is a primary or regraft recipient based on prior transplantation. Data collected for SAB testing include vendor of the SAB reagent; serum pretreatment; raw or normalized HLA antibody median florescence intensity (MFI), reported by the participating laboratory based on their clinical practice; and calculated panel reactive antibodies (CPRAs). Data collected in the VXM are cPRA, number and strength of HLA DSA, DSA characterization as specific for a repeat mismatched HLA antigen from the patient's previous transplant, and a renal transplant risk assessment for each virtual donor–recipient pair (acceptable, unacceptable).

In Phase II, participating laboratories receive 4 recipient serum samples for SAB testing. The laboratories will then perform FXM using lymphocytes from 2 donors provided against the 4 sera. The unique feature of the UCLA VXM Exchange survey is that 1 recipient–donor pair in Phase II was sent as a blinded sample to the participating laboratory from the VXM survey from Phase I for FXM testing, allowing comparison between FXM and VXM (Figure 1). Data collected for Luminex SAB testing include vendor of the SAB reagent, serum pretreatment, MFI values of HLA antibodies, and CPRAs. Data collected for FXM include negative control median channel, positive cutoff, pronase treatment, median channel shift (MCS), and HLA DSA. Research approval for this study was granted by the UCLA Institutional Review Board (IRB#10-001689).

### Consensus Evaluation

FXMs are graded based on the consensus of a minimum of 10 laboratories and 80% agreement among laboratories reporting positive/negative assignments for the FXM. SAB is graded based on 90% agreement among laboratories reporting HLA class I/class II antibody specificities. An individual laboratory's results falling within the consensus was graded as satisfactory. Results reported as borderline or undetermined were not graded. The VXM was designed as an educational exercise, and as such, virtual predictions of the expected FXM were not graded. All SAB, VXM, and FXM results were reported through the website https://cell-exch.ctrl.ucla.edu/.

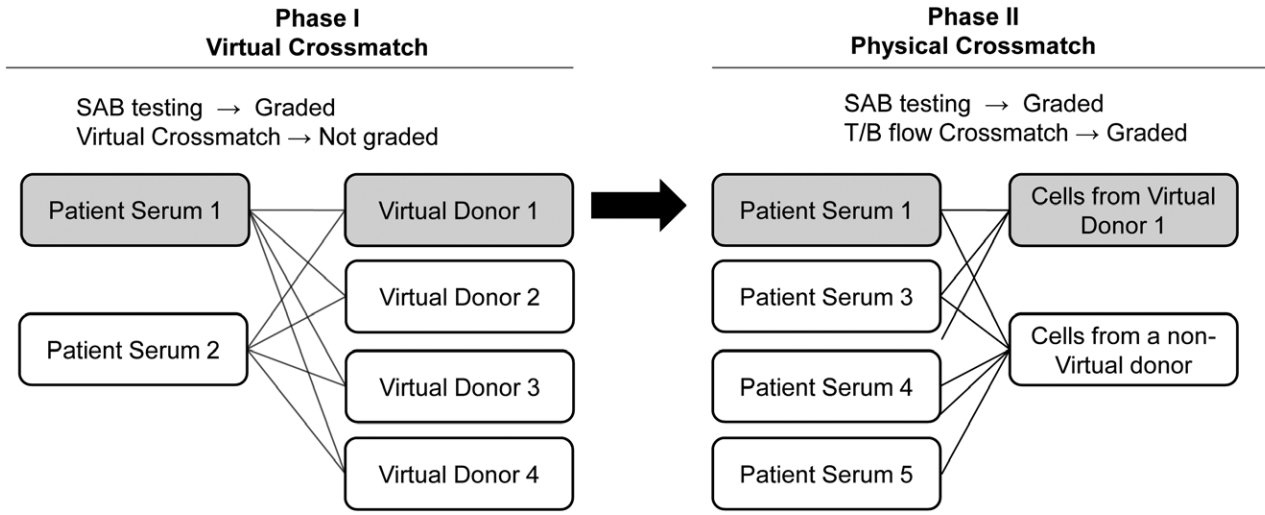



**FIGURE 1.** UCLA VXM Exchange flow chart. UCLA VXM exchange consist of 2 phases. In phase I, 2 serum samples and 4 virtual donors are provided to the laboratories to perform SAB tests and 8 VXM. In phase II, 4 recipient serum samples and lymphocytes from 2 donors are provided to perform SAB tests and 8 FXMs. In phase II, 1 recipient–donor pair will be selected from the VXM of phase I; therefore, a direct comparison between FXM and VXM can be achieved. FXM, flow cytometry crossmatch; SAB, single antigen bead test; UCLA, University of California, Los Angeles; VXM, virtual crossmatch.

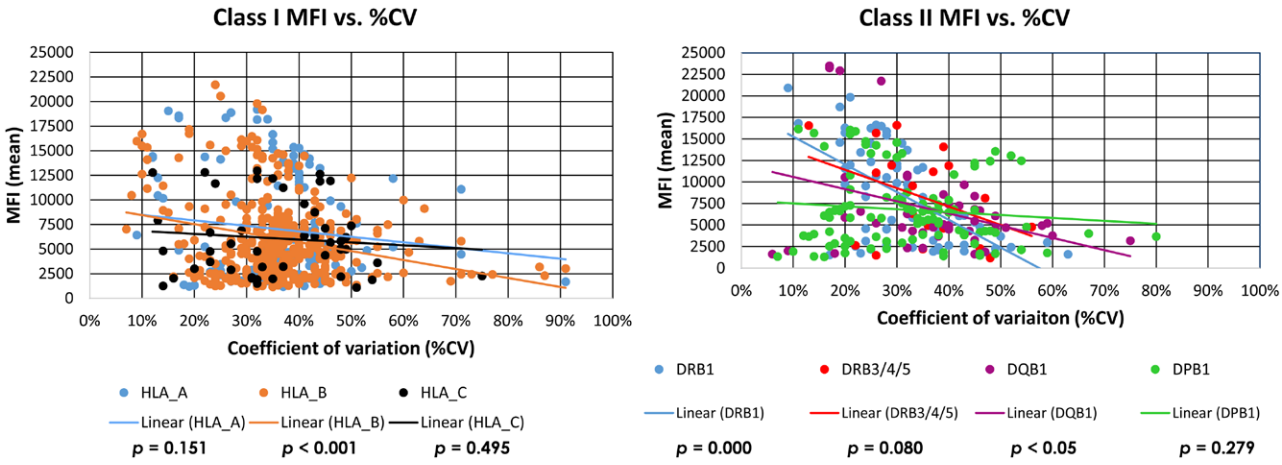



**FIGURE 2.** Interlaboratory variability in the reporting of MFI values. Interlaboratory variability (%CV) in the reporting of MFI values for class I and class II antibodies for Exchanges 6 to 17. A total of 12 sera were examined twice across the 13 exchanges. MFI values for the highest DSA with MFI >1000 and reported by a minimum of 5 laboratories were plotted against %CV to illustrate the variability observed among laboratories in the reporting of MFI values. %CV, coefficient of variation; DSA, donor-specific antibody; MFI, median florescence intensity.

### Statistics and Data Analysis

This is an observational study of sera screen and cell crossmatch results reported by laboratories to the UCLA VXM Exchange Program. Continuous data including MFI and MCS were summarized using means, SDs, and coefficients of variations. Categorical prediction outcomes were summarized as frequencies and percentages. Data were also displayed graphically using scatterplots and bar charts for continuous and categorical outcomes, respectively. To assess linear associations between MFI and MCS, Pearson's squared correlation coefficients ($R^2$) were obtained from linear regression analysis. Bland-Altman plot was used to assess the variance of SAB MFI. Concordance in positive VXM predictions was assessed by the percent agreement in positive reports from all possible actual outcomes (ie, positive, negative, or not done). Overall accuracy between the 2 methods was estimated by the area under the receiver operator curve (AUC). The 95% confidence intervals were derived assuming asymptotic normality. Data were analyzed using STATA (StataCorp, 2021, Stata Statistical Software: Release 17, College Station, TX), and *P* values were 2-sided with a value <0.05 considered statistically significant.

## RESULTS

Thirty-five laboratories participated in the UCLA VXM Exchange survey during a 5-y period from 2014 to 2019. A total of 72 well-defined reference serum samples and 36 donor blood samples were sent out to participating laboratories, resulting in a total of 144 T/B-FXM pairs from Exchanges 1 to 18. The VXM survey was initiated in Exchange 6, resulting in a total of 112 T/B-VXM pairs from Exchanges 6 to 17. There were 1992 T-cell FXM (T-FXM), 2112 B-cell FXM (B-FXM), and a total of 1604 T-cell VXM (T-VXM) and 1658 B-cell VXM (B-VXM) completed.

### SAB Interlaboratory Variability

We first examined the inter- and intralaboratory variability of SAB tests. Commercial SAB kits from 2 manufacturers were used among laboratories without standardized protocols. The majority of laboratories (84%) used SAB reagents from vendor 1, whereas the remainder (16%) used reagents from vendor 2. The positive cutoff ranged from 700 to 3000 MFI for HLA-A, HLA-B, HLA-DRB, and HLA-DQB1 loci and 700 to 15 000 MFI for HLA-C, HLA-DPB1, and HLA-DQA1 among labs. To illustrate the interlaboratory variability in SAB assay, coefficients of variation (%CV) of SAB MFI values were plotted against mean MFIs for 12 serum samples tested in Exchange 7 to 18 (Figure 2). Interlaboratory variability in the reporting of class I and class II antibody strength is plotted separately. In general, variability between laboratories decreased as MFI ranges for all HLA loci increased. For HLA-A and HLA-B locus antibodies, for example, %CV generally fell below 40% for antibodies with >15 000 MFI. In the case of DRB- and DQB1-locus antibodies, %CVs fell below 30% for antibodies with >15 000 MFI. For both class I and class II, a majority of antibodies >5000 MFI had %CV <50%.

Interfering substances present in patient serum have been reported to cause falsely decreased MFI values in SAB tests, particularly in highly sensitized patients.[18] Serum pretreatment with ethylenediaminetetraacetic acid (EDTA) or dithiothreitol (DTT) has been reported to reduce serum interference[19,20] and is recommended by the Sensitization in Transplantation: Assessment of Risk Workgroup.[21] Of the 20 laboratories reporting serum treatment, 6 used EDTA, 3 used DTT, 1 used fetal bovine serum, 1 used both DTT/fetal bovine serum, and 9 did not use any treatment. Interlaboratory comparison of antibodies reported that a single serum sample tested across 2 consecutive exchanges showed less variability among laboratories in the reporting of antibody strength for treated serum than for untreated serum. Laboratories that treated the sera before testing showed 10% to 15% less deviation in MFI values across repeated testing compared with laboratories that did not treat the sera. For antibodies ranging between 10 000 and 22 000 MFI, %CVs for the treated serum were between 8% and 24% (Figure 3A). For the untreated serum, %CV values were higher at 27% to 40% (Figure 3B).

### SAB Intralaboratory Variability

To understand the intralaboratory variability in SAB testing, we analyzed a total of 12 samples that were tested twice by the same laboratory across 13 exchanges

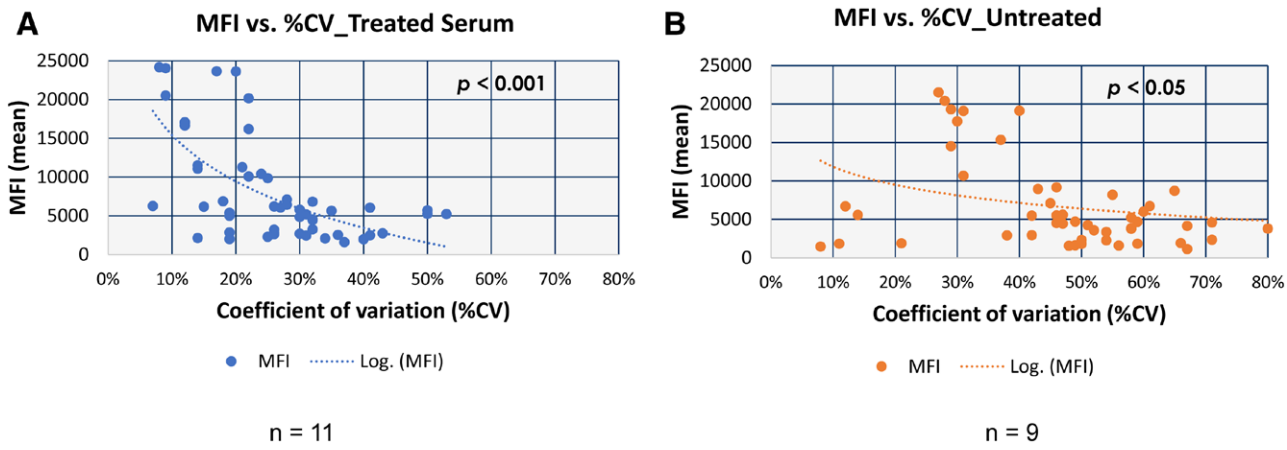



**FIGURE 3.** A comparison of interlaboratory variability for treated vs untreated serum. Comparison of interlaboratory variability (%CV) in treated (n = 11) vs untreated (n = 9) samples. The same serum sample was tested by SAB twice across 2 consecutive exchanges among laboratories. Antibody strength (mean MFI) was plotted alongside %CV for an untreated and a treated serum sample. For the treated serum (A), interlaboratory variability was between 8% and 24% CV for antibodies with >10 000 MFI. For the untreated serum (B), interlaboratory variability ranged between 20% and 40%. %CV, coefficient of variation; MFI, median florescence intensity; SAB, single antigen bead test.

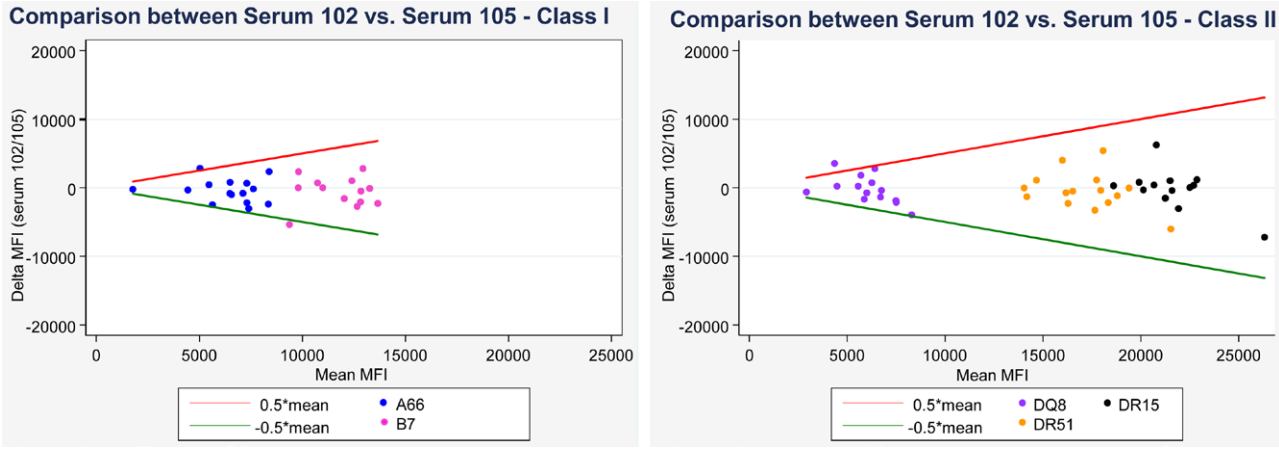



**FIGURE 4.** Intralaboratory variability on repeated serum samples. Each point on the Bland-Altman plot represents the change (Δ) in the mean MFI reported for individual laboratories for a serum sample tested 2 times across consecutive exchanges. The boundaries on the plots represent the expected variation of the mean value reported for antibodies present in the serum. The position of the points between the 2 boundaries shows MFI for the highest antibodies tested a second time to fall within a variation of 50% of the mean value. MFI, median florescence intensity.

(Exchanges 6–18, Figure 4). Overall, the reporting of antibody specificities and strengths for each repeated serum was consistent for individual laboratories with their previously obtained value. Mean MFI for highest MFI antibodies tested a second time fell within 50% of initial MFI values, as shown in the figure within the boundaries. For antibodies >15 000 MFI, the boundaries were narrower, which agrees with previous reporting by Reed et al.[22]

## Interlaboratory FXM Agreement

Among the 144 donor–recipient pairs, the agreement in reporting of physical FXM results was similar between the T-FXM and the B-FXM, with 86% (124/144) of T-FXM achieving ≥80% agreement compared with 84% of (121/144) B-FXM. In the 20 of 144 T-FXMs that failed to reach ≥80% consensus, 17 of 20 were observed in the presence of median class I DSA <7000 MFI, and 3 of 144 were observed in the absence of class I DSA, implicating false-positive FXM. All 23 of 144 B-FXMs that failed to reach ≥80% consensus occurred when the class I or class II DSA was <14 000 MFI. DSAs to HLA loci have a lower surface expression level on lymphocytes such as C and DP, as indicated in Exchanges 12 and 15, and also played a role in the variability observed in the sensitivity of the B-FXM.

## Correlation of FXM With HLA DSA Strength

The correlation of T- and B-FXM results with DSA detected by SAB testing is critical for VXM prediction. To examine the relationship between FXM and DSA strength, mean MFI values of highest DSA were plotted alongside median FXM results for each T-FXM and B-FXM of survey 1 to 18 (Figure 5A and B). Figure 5A illustrates the correlation of highest HLA class I DSA MFI on T-lymphocyte FXM (n = 144). A linear correction was observed between T-FXM and MFI of the highest titer class I DSA ($R^2 = 0.8692$, $P < 0.001$). In general, positive T-FXMs were found in the presence of the highest class I DSA with MFI >3000. As MFI values rose, so did T-FXM MCS values. The correlations of the average of class I DSA (Figure S1,

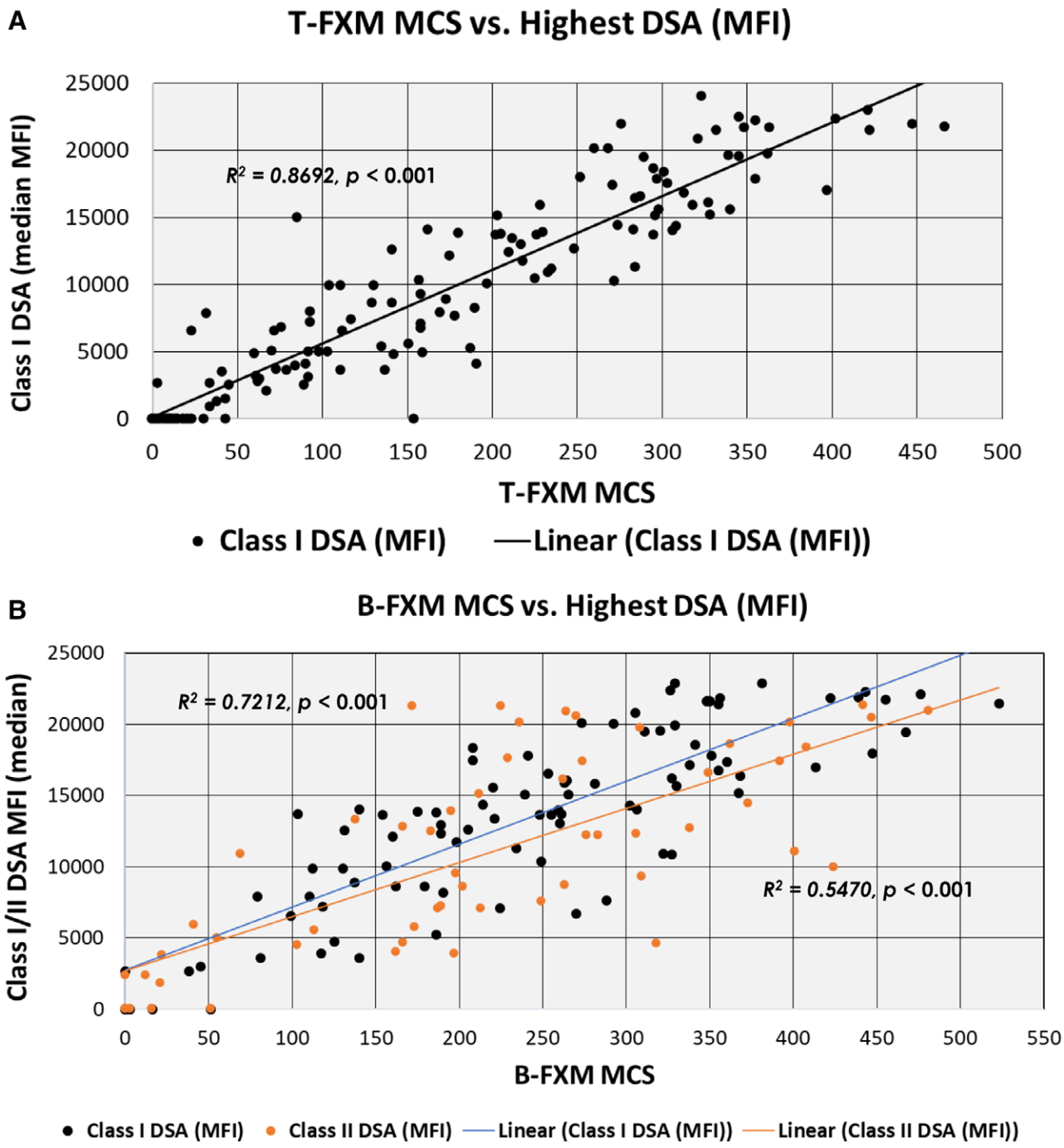



**FIGURE 5.** Correlation of highest DSA with flow crossmatch. A, Scatter plot illustrating the positive linear relationship between highest class I DSA MFI and T-FXM MCS values. Each data point (MCS, MFI) represents a single T-FXM examined from Exchange 1 to 18 (n = 144). The correlation between highest HLA class I DSA MFI vs T-FXM MCS showed an $R^2$ of 0.8692, $P < 0.001$ among laboratories. B, Scatter plot illustrating the positive linear relationship between highest class I/II DSA MFI and B-cell FXM MCS values. Each data point (MCS, MFI) represents a single B-FXM examined from Exchange 1 to 18 (n = 144). The correlation between highest HLA class II DSA MFI vs B-FXM MCS showed an $R^2$ of 0.6228, $P < 0.001$ among laboratories. DSA, donor-specific antibody; FXM, flow cytometry crossmatch; MCS, median channel shift; MFI, median florescence intensity; T-FXM, T-cell FXM.

http://links.lww.com/TP/C731) and the additive of class I DSA (Figure S2, http://links.lww.com/TP/C731) with T-FXM have an $R^2$ of 0.7685 ($P < 0.001$) and 0.7278 ($P < 0.001$), respectively, suggesting that the higher DSA MFI correlates better with T-FXM.

Figure 5B illustrates the correlation of the highest class I and/or class II DSA strength on B-FXM (n = 144). In general, positive B-FXMs were observed in the presence of DSA >5000 MFI. The correlation between highest class I DSA with B-FXM ($R^2$ = 0.7212, $P < 0.001$) has better correlations than the correlation between the highest class II and B-FXM ($R^2$ = 0.5470, $P < 0.001$). The correlations of average and additive class I/II DSA with B-FXM were poor (average of DSA, $R^2$ = 0.5583, $P < 0.001$; Figure S3, http://links.lww.com/TP/C731; additive of DSA, $R^2$ = 0.5575, $P < 0.001$; Figure S4, http://links.lww.com/TP/C731).

For both T- and B-FXM, a positive linear relationship was still observed; and the FXM results increased as DSA strength increased. This resulted in improved agreement among laboratories in the reporting of positive B-FXM outcomes in the presence of strong DSA. Unexpectedly strong B-FXM >400 MCS was observed in the presence of moderate DSA (C15 at 4985 ± 1775 MFI, DQ7 at 9978 ± 6649 MFI). Saturation and/or prozone effects were present in the sample because the dilution of the DTT-treated serum by the reference laboratory showed C15 at 3537 MFI and DQ7 at 13 862 MFI. Eleven of 12 laboratories reported strong positive B-FXM despite pronase treatment suggesting pronase treatment had no effect in correcting prozone phenomenon.

### Concordance Between VXM and FXM

For the VXM, 91 of 112 (81%) T-VXMs and 90 of 112 (80%) B-VXMs achieved >80% agreement on VXM prediction based on the absence or presence of HLA DSA among laboratories. HLA class I and/or class II DSA were present in all but 5 of the 144 donor–recipient combinations. A direct comparison of VXM predictions with FXM outcome is shown in Table 1. For the T-VXM, 89 of 112 (79%) positive predictions matched the physical T-FXM. For the B-VXM, 117 of 135 (87%) positive predictions agreed with the physical B-FXM. Receiver operating characteristics analyses showed similar concordance by AUC values of T cell (AUC = 0.72, 95% CI of 0.63, 0.81) and B cell (AUC = 0.67, 95% CI of 0.58, 0.76), respectively.

To provide a better understanding of the relationship between VXM and FXM, DSA and MFI values for the 12 VXMs completed in this study are shown in Table 2 and illustrated in Figure 6. In addition, for each laboratory, concordance rates (% agreement between VXM predictions and the FXM) are shown alongside each challenge in Table 2. Overall, concordance between predicted positive T-FXM outcome and the physical T-FXM was >80% when class I DSAs were >3000 MFI. Concordance between predicted positive B-VXM and the B-FXM appeared to only rise above 80% in the presence of the highest DSA >12 000 MFI. High expressing DP5 DSA[23] were sent out to understand the correlation of DP DSA with FXM. However, despite the presence of strong DSA to DP5 at 16 024 ± 3299 MFI reported by all 16 laboratories, the median B-FXMs were borderline positive with 2 of 16 laboratories reported negative FXM results.

## DISCUSSION

Accurate assessment of antibodies to donor HLA antigens is crucial for donor selection and guiding immunotherapy. There has been a significant increase in the use of VXM at the time of deceased donor allocation since the advent of SAB-based antibody testing and molecular-based HLA typing of all loci. The accuracy of VXM prediction will be further advanced when high resolution, allele level HLA typing is available, particularly for patients who display allele-specific HLA antibodies. Puttarajappa et al[11] reported that the use of VXM has increased at 2.4% per year since 2014 and that 18% of deceased donor transplants were based solely on VXM in 2018. They demonstrated that proceeding to transplantation based on VXM alone had no negative impact on kidney transplant outcomes. Currently, how the VXM is performed is based on the agreement between the transplant centers with their supporting HLA laboratories.

Despite the fact that participating laboratories used different standard operating procedures and reagents from different manufacturers, approximately 80% concordance between the VXM predictions and the physical FXM was achieved in the presence of HLA DSA. Our VXM Exchange showed that the variability of SAB tests is relatively small for HLA antibodies >10 000 MFI, but for antibodies below 5000 MFI, a larger variation was observed. Reed et al[22] demonstrated that, by standardizing SAB reagents and standard operating procedures, MFI variation could be reduced from 62% to 25%. This suggests that standardization among laboratories will be needed to improve the VXM accuracy and downstream patient management.

Although negative VXM prediction for nonsensitized patients is fairly accurate,[14] the accuracy of VXM prediction in the presence of HLA DSA can be complicated by the patient's sensitizing events, DSA titer, DSA loci, the number of DSAs, interfering substances in the patient's serum, and intrinsic differences between solid-phase assays and cell-based assays. On cellular surfaces, HLA molecules are able to move and twist to create spaces allowing interlocus antibodies (eg, A2 and B57) that share the same epitope to bind; however, this is not possible on a fixed surface in the SAB assay. Another intrinsic difference between SAB and FXM is that differential peptide loading contributes to differences in HLA antigen and antibody binding affinities and avidities in vivo.[24,25]

**TABLE 1.**

**Direct comparison of physical crossmatch results vs virtual crossmatch results for Exchanges 6 to 17**

| | | T-cell physical XM | | | | | B-cell physical XM | | |
|---|---|---|---|---|---|---|---|---|---|
| | | + | – | Ø | | | + | – | Ø |
| Virtual T-cell flow XM | + | 89 | 13 | 11 | Virtual B-cell flow XM | + | 117 | 19 | 15 |
| | – | 16 | 44 | 5 | | – | 15 | 16 | 8 |
| | Ø | 7 | 7 | 1 | | Ø | 2 | 1 | 0 |
| | | 112 | 64 | 17 | | | 134 | 36 | 23 |

+: positive; –: negative; Ø: borderline/undetermined/not reported. The total sum of true positive (+, +) T-FXM predictions (n = 89) (ie, predictions matching T-FXM outcome) and true positive (+, +) B-FXM predictions (n = 117) are shown. T-cell: POS concordance = 79% (95% CI, 71%-86%); AUC = 0.72 (95% CI, 0.63-0.81). B-cell: POS concordance = 87% (95% CI, 71%-84%); AUC = 0.67 (95% CI, 0.58-0.76).

AUC, area under the curve; CI, confidence interval; FXM, flow cytometry crossmatch; POS, positive; T-FXM, T-cell FXM.

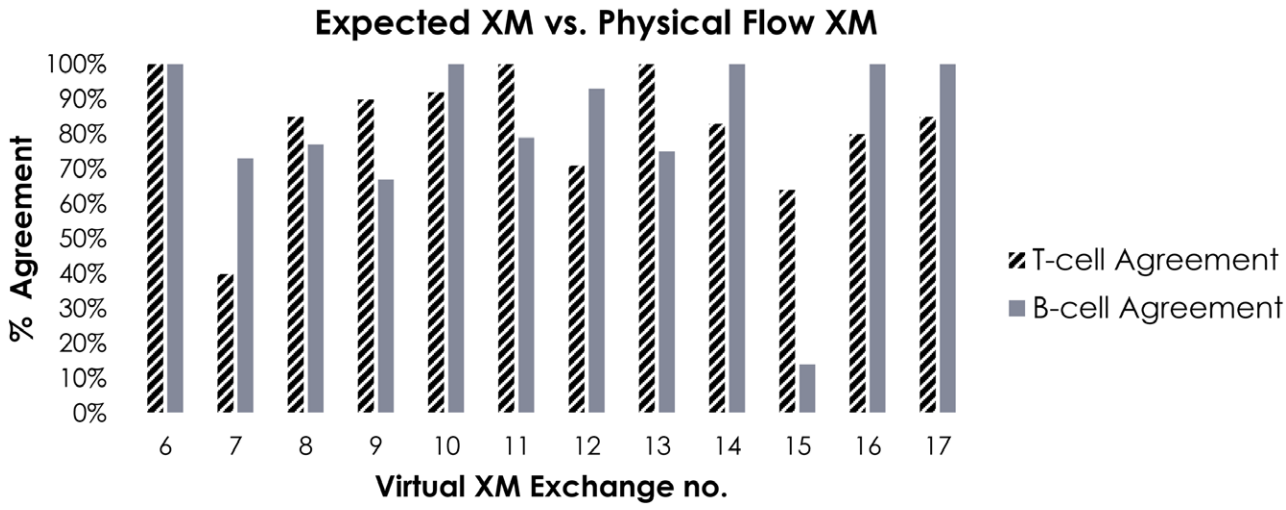
**TABLE 2.**

**Direct comparison of the VXM vs the physical crossmatch**

| | VXM | | | | Physical flow crossmatch | | | | Concordance | |
|---|---|---|---|---|---|---|---|---|---|---|
| Exchange no. | Class I DSA (MFI) | T-cell prediction | Class II DSA (MFI) | B-cell prediction | Class I DSA (MFI) | T-cell result | Class II DSA (MFI) | B-cell result | T cell | B cell |
| 6 | B48 (17 978 ± 2811), Cw8 (2715 ± 626), A68 (1138 ± 498) | POS (5) | No DSA | POS (5) | B48 (16 738 ± 2967), Cw8 (3316 ± 2222) | POS (5) | No DSA | POS (5) | 100% | 100% |
| 7 | B41 (2127 ± 854) | NEG (7)<br>POS (4) Borderline (1) | DP4 (3629 ± 686) | POS (8)<br>NEG (4) | B41 (2162 ± 568) | POS (10)<br>NEG (1)<br>U (1) | DP4 (4703 ± 1765) | POS (11)<br>U (1) | 40% | 73% |
| 8 | A11 (3249 ± 1180) | POS (13)<br>NEG (2) | DQ7 (5215 ± 1272) | POS (13)<br>NEG (1) | A11 (2963 ± 1242) | POS (11)<br>NEG (3)<br>U (1) | DQ7 (4496 ± 1736) | POS (10)<br>NEG (4) | 85% | 77% |
| 9 | A1 (3450 ± 827), Cw7 (3004 ± 573) | POS (10)<br>Borderline (2) | No DSA | POS (9)<br>NEG (2)<br>Borderline (1) | A1 (2738 ± 940), Cw7 (2689 ± 597) | POS (9)<br>NEG (3) | No DSA | POS (6)<br>NEG (6) | 90% | 67% |
| 10 | B18 (7160 ± 2586), Bw6 (11 236 ± 5526) | POS (13)<br>NEG (1) | No DSA | POS (10)<br>NEG (4) | B18 (7134 ± 2572), Bw6 (12 230 ± 5720) | POS (12)<br>NEG (2) | No DSA | POS (10)<br>NEG (4) | 92% | 100% |
| 11 | B27 (2843 ± 1321) B51 (4650 ± 1572) | POS (14) | DR53 (11 186 ± 4004) | POS (14) | B27 (2889 ± 1060), B51 (4546 ± 1597) | POS (14) | DR53 (11 679 ± 3998) | POS (11)<br>NEG (3) | 100% | 79% |
| 12 | Cw5 (2116 ± 621) | NEG (10)<br>Borderline (3)<br>U (1) | No DSA | NEG (13)<br>Borderline (1) | Cw5 (2492 ± 1127) | NEG (14) | No DSA | NEG (14) | 71% | 93% |
| 13 | Cw4 (3215 ± 1151) A23 (13 609 ± 5611) Cw7 (12 623 ± 5423) | POS (16) | No DSA | POS (16) | A23 (12 276 ± 4804) Cw7 (11 940 ± 5327) | POS (16) | No DSA | POS (12)<br>NEG (2)<br>Borderline (2) | 100% | 75% |
| 14 | B58 (4363 ± 1568) | POS (15)<br>Borderline (4) | DR4 (11 386 ± 3762)<br>DR53 (4629 ± 1744)<br>DQ4 (3221 ± 1390) | POS (19) | B58 (4984 ± 2265) | POS (18)<br>NEG (1) | DR4 (12 338 ± 3073)<br>DR53 (4985 ± 1747)<br>DQ4 (5198 ± 2955) | POS (19) | 83% | 100% |
| 15 | No DSA | NEG (14)<br>POS (1)<br>Borderline (1) | DP5 (16 024 ± 3299) | NEG (14)<br>POS (1)<br>Borderline (1) | No DSA | NEG (9)<br>POS (5)<br>Borderline (2) | DP5 (13 295 ± 3951) | POS (13)<br>NEG (2)<br>Borderline (1) | 64% | 14% |
| 16 | No DSA | NEG (15) | DR17 (14 056) DR52 (8229) DP2 (2904) | POS (15) | No DSA | NEG (12)<br>Positive (1)<br>Borderline (2) | DR17 (13 689 ± 4299)<br>DR52 (11 878 ± 4582)<br>DP2 (4112 ± 1955) | POS (15) | 80% | 100% |
| 17 | No DSA | NEG (13)<br>Borderline (1) | DQ6 (22 903 ± 4271)<br>DQA1 (19 059 ± 7466) | POS (14) | No DSA | NEG (11)<br>Borderline (2)<br>Positive (1) | DQ6 (21 897 ± 4715) | POS (14) | 85% | 100% |

Direct comparison of VXM predictions with FXM outcomes for Exchanges 6 to 17. Expected FXM predictions, along with DSA and DSA strengths, are shown on the left-hand side of the table for an individual donor cell–recipient serum pair. Actual physical FXM outcome for the same cell-serum pair are shown on the right for comparison. Agreement between predictions and the physical FXM is shown to be influenced by DSA strength.

DSA, donor-specific antibody; FXM, flow cytometry crossmatch; MFI, median florescence intensity ± SD; NEG, negative; POS, positive; U, undetermined; VXM, virtual crossmatch.




## Reported DSA and MFI strength

| Virtual XM Exchange | class I DSA | MFI | class I DSA | MFI | class I DSA | MFI | class II DSA | MFI | class II DSA | MFI | class II DSA | MFI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | B48 | 17978 | Cw8 | 2715 | A68 | 1138 | none | 0 | - | 0 | - | - |
| 7 | B41 | 2127 | - | - | - | - | DP4 | 3629 | - | - | - | - |
| 8 | A11 | 3249 | - | - | - | - | DQ7 | 5215 | - | - | - | - |
| 9 | A1 | 3450 | Cw7 | 3004 | - | - | none | 0 | - | - | - | - |
| 10 | Bw6 | 11236 | B18 | 7160 | - | - | none | 0 | - | - | - | - |
| 11 | B51 | 4650 | B27 | 2843 | - | - | DR53 | 11186 | - | - | - | - |
| 12 | Cw5 | 2116 | - | - | - | - | none | 0 | - | - | - | - |
| 13 | A23 | 13609 | Cw7 | 12623 | Cw4 | 3215 | none | 0 | - | - | - | - |
| 14 | B58 | 4363 | - | - | - | - | DR4 | 11386 | DR53 | 4629 | DQ4 | 3221 |
| 15 | none | 0 | - | - | - | - | DP5 | 16024 | | | | |
| 16 | none | 0 | - | - | - | - | DR17 | 13416 | DR52 | 11920 | DP2 | 3819 |
| 17 | none | 0 | - | - | - | - | DQ6 | 22903 | DQA1 | 19059 | | |

**FIGURE 6.** Comparison of VXM prediction and actual physical crossmatch. Each bar represents the percent agreement between VXM predictions and the physical FXM for Exchanges 6 to 17 (n =112). DSAs reported for each donor in an exchange, along with reported mean MFI, are shown in the accompanying table to illustrate the influence of DSA strength on the accuracy of predictions. DSA, donor-specific antibody; FXM, flow cytometry crossmatch; MFI, median florescence intensity; VXM, virtual crossmatch.

In addition, the MFI values of antibodies against public epitopes such as Bw4/Bw6 antibodies may be underestimated in a SAB test because of the dilution effect. False-negative VXM prediction has been reported in patients that seemly display "weak" Bw4 and Bw6 antibodies.[26] Therefore, FXM serves as an important tool for patients that display multiple weak HLA DSA to understand the additive effects of these DSAs. In addition, discordance between FXM and VXM can also be caused by variable HLA antigen expression of donor cells, particularly for HLA-C,[27,28] HLA-DQ,[29] and HLA-DP[23] antigens. For example, discrepant B-FXM results were reported in our exchange despite the presence of strong DP DSA over 15 000 MFI shown in Figure 6. Moreover, Badders et al[30] showed that the expression of HLA class I was similar on B cells isolated from living donor blood and spleen/nodes of deceased donors, but the expression was significantly lower on B cells isolated from deceased donor blood, whereas the HLA class II expression was significantly higher in B cells isolated from living donors than in B cells isolated from deceased donors.[30]

Complement/IgM interference or prozone due to HLA antibody access in the patient's serum can result in significant lower MFI in SAB tests and false-negative VXM.[19,31,32] The interference of IgM/complement can be abrogated by pretreating the serum with EDTA, DTT, C1 inhibitors, or serum dilution.[19] We showed that laboratories that used serum pretreatment had lower inter- and intralaboratory variability than those that did not pretreat the serum. The inhibition of antigen-antibody binding in the SAB test caused by antibody excess was demonstrated by Sullivan et al.[20] The authors successfully abolished the prozone effect by using a secondary antibody that conjugated to biotin and then probed with PE-conjugated streptavidin. The authors hypothesized that the biotinylated secondary antibody is less likely to hinder its binding to HLA antibodies because of its low molecular weight. In addition, the long spacer between biotin and streptavidin allows a secondary antibody to have room to bind to the HLA antibody. Antibody excess is reported to be more common in highly sensitized patients. Jani et al[12] reported that 8.7% of patients waiting for a kidney transplant with CPRA <80% exhibited a prozone effect, compared with 25% in patients with CPRA between 80% and 97%, and 41.7% in patients with CPRA ≥98%. The authors also demonstrated that the prozone effect is more

common for HLA-A and HLA-B loci antigens than for HLA-C, HLA-DR, and HLA-DP loci antigens. The best way to eliminate the prozone effect caused by antibody excess is dilution of the patient serum. However, the dilution factor can be difficult to determine because the antibody titer is unknown. Tambur et al[14] demonstrated that C1q-SAB results correlated best with the IgG-SAB test with titration, suggesting C1q-SAB can be utilized as 1 single test to identify prozone effect efficiently. However, because C1q-SAB is unable to detect weak HLA antibodies[18] and does detect IgM antibodies, C1q-SAB needs to be run in parallel with the SAB test.

Other limitations of SAB tests include false-positive reactivity due to cryptic epitopes and limited HLA alleles in the current SAB panels. Denatured HLA antigens on a SAB bead assay could cause false-positive VXM prediction. Because SAB beads have recombinant HLA antigens bound by hydrophobic interactions, the conformation of HLA molecules present on the surface of beads could be different from their native conformation on the cell surfaces. This can expose cryptic epitopes on HLA molecules that result in nonspecific antibody binding.[12,17] HLA is the most polymorphic gene complex in the human genome. When a donor carries HLA alleles that are not represented in the SAB panel, the VXM prediction is difficult. In this case, FXM serves a useful tool for risk stratification between the donor–recipient pair.[33] Wehmeier et al[34] showed that current SAB panels cover ~98.5% of HLA eplets; however, the HLA alleles in minority populations remain underrepresented. Kumar et al[35] showed that, among 966 subjects, they typed at high resolution, 811 subjects (83.95%) had at least 1 class I allele unrepresented in the SAB panels, and 809 subjects (83.75%) had at least 1 class II allele unrepresented in the SAB panels in India. In the United States, Zavyalova et al reported that ~15% of patients awaiting transplantation display allele-specific HLA antibodies using extended SAB panels.[15] Therefore, the use of SAB with broader HLA allele coverage will increase the accuracy of VXM at the time of donor offer.

Despite the limitations of the SAB assay, we demonstrated that, with better understanding of the variability of HLA antigen expression and prozone mitigation strategies, the accurate prediction of HLA DSA with FXM can be achieved at the time of organ offers for most cases. However, FXM as a direct risk assessment between a patient and a particular donor still serves an important tool, particularly in highly sensitized patients, to help clinicians make decisions on pretransplant and posttransplant management. Risk assessment for the UCLA VXM survey is not graded because of high practice variability among transplant centers. Whether a transplant center would like to transplant across HLA DSA depends on the risk tolerance of the program and clinical status of the patient. It also depends on if desensitization therapies such as plasmapheresis, IVIg,[36] rituximab,[37] bortezomib,[38] eculizumab,[39] or iDES[40] are available to patients.

In summary, the UCLA VXM Exchange provides important information to better understand the variability/limitations of SAB, FXM testing, and VXM prediction in the presence of HLA DSA and allows laboratories to compare their practice with other centers. For recipient sera containing multiple weak DSAs with a potential for an additive effect, or rare donor alleles not covered by currently manufactured SAB panels, FXM remains a valuable tool to determine the compatibility between donors and recipients. With the increasing use of the VXM, educational activities should continue to provide quality control for more unified practices on VXM to ensure accurate and consistent risk assessment for organ transplantation.

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