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Perception Versus Reality?: Virtual Crossmatch—How to Overcome Some of the Technical and Logistic Limitations

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Abstract

The goal of this work was to evaluate concordance between (a) actual flow cytometric crossmatch (FCXM) that is performed by the OPO laboratory servicing our transplant center and (b) virtual XM (vXM) prediction based on antibody identification by solid-phase methods performed in our laboratory.

A total of 1586 FCXM, performed between June 2007 and September 2008, between all potential deceased donors in our region and sera from patients awaiting kidney or kidney–pancreas transplant, listed at Northwestern Memorial Hospital were evaluated.

A key finding of this analysis was the understanding that a thorough vXM cannot be performed in some donor/recipient pairs due to the lack of certain antibody profile data specific to the donor in question. Obtaining more in depth and stringent information regarding antibody specificities, we demonstrate an excellent sensitivity and specificity of the vXM assays—86.1% and 96.8%, respectively, with a positive likelihood ratio and negative likelihood ratios of 26.9 and 0.14, respectively.

The vXM can serve as an outstanding tool to predict HLA compatibility between donor and recipient, with the caveat that the presence/absence of all antibodies against the potential donor and their strength have been thoroughly investigated.

Keywords

Antibodies; antibody-mediated rejection; crossmatching; HLA; kidney transplantation

Introduction

The introduction of solid-phase-based methods for detecting anti-HLA antibodies has been a significant technical advance that has increased the specificity and sensitivity of detecting antibodies directed against HLA class I and class II antigens (1,2). Some concerns, however, have been expressed regarding the utility of applying these tests as a method to predict a 'true positive' actual crossmatch (XM) in the clinical scenario. Several studies, including abstracts from scientific meetings, suggest that disparate sets of guiding principles have been applied by different laboratories to define the presence of HLA-specific antibodies (3–6). An attempt to develop consensus guidelines through meetings between representatives of the various transplant-related disciplines has not been achieved (7).

The improved ability to define HLA specificity has advanced the concept of defining a calculated panel-reactive antibody (cPRA; (8)) assessment. Yet the aforementioned issues have led to concerns as to its reliability when logically extended to the virtual crossmatch (vXM) concept, to enhance the timely allocation of organs to compatible recipients (9–16). In fact, many transplant professionals are reluctant to approve of changes in UNOS policies that would advance the use of cPRA and vXM methodology on regional and national levels (17). We propose that much of this resistance is due mainly to lack of information on two accounts: (a) antibody analysis is often incomplete, some donor-specific information may be unintentionally ignored or remain uninterpreted; (b) the presence of donor-specific antibodies (DSA) should be considered not as a simple yes/no response, rather information regarding the antibody strength as well as the number and level of expression of the relevant epitopes should be considered.

To further interrogate this hypothesis, we evaluated the concordance between actual flow cytometric XM (FCXM) performed by the OPO laboratory servicing our transplant center and the vXM prediction based on antibody identification by solid-phased methods performed in our laboratory. All XM assays were performed using cells obtained from deceased donors. Importantly, discrepancies between the two XM results were thoroughly evaluated and when possible additional work was done to resolve or explain the differences in results.

Material and Methods

Actual FCXM were performed by the local OPO laboratory. A total of 1586 FCXM were performed during the period between June 2007 and September 2008 between all potential deceased donors in our region and sera from patients awaiting kidney or kidney–pancreas transplant, listed at Northwestern Memorial Hospital. FCXM assays with T-cell positive, B-cell negative results (6.7% of all FCXM) were excluded from this study assuming potential technical problems in executing the FCXM since HLA antigens are present both on T- and B-cells and a positive T-cell XM—if due to HLA antibodies—should be accompanied with a positive B-cell XM. Thus, all positive FCXM assays were either T-cell and B-cell positive, or T-cell negative B-cell positive FCXM. A total of 1480 FCXM assays were used for final evaluation. This number of assays corresponded to FCXM performed between 461 potential recipients and 268 deceased donors. Fifty-one percent of the patients were male; 34% had

PRA <20% against both class I and class II; 31% had PRA of 20–80%; and 35% had PRA >80% against either class I or class II antigens. Most sensitized patients had antibodies to both class I and class II antigens (68%), 29% had antibodies to HLA class I antigens only, and 3% had antibodies to HLA class II antigens only.

Detection of HLA-specific antibodies

All patients underwent screening for HLA-specific IgG antibodies prior to listing as potential kidney or kidney–pancreas recipients using the flow-PRA screening assay. Individuals with positive PRA were further evaluated using the flow-PRA-specific or flow-PRA single antigen kits for HLA class I or class II specificities, tailoring the testing protocol for each patient as previously described (18). Of note, our routine practice calls for the use of a limited panel of antigen-coated beads for the flow-PRA class I single antigen testing, those antigens that are more frequent among the donor population, to increase cost effectiveness. Antigens in the limited panel are considered to be more frequent in the population and include 32 of the total 80 reagents available for extended class I antibody identification. All flow-PRA reagents were purchased from One Lambda, Canoga Park, CA.

In certain cases, where % PRA did not correlate with identified HLA antibody makeup, additional assays were performed utilizing the complete reagent set for class I antibody identification, as we have previously described (18). This second level of testing was designed to detect antibodies against antigens that are less common among the UNOS donor population, as well as antibodies against HLA-Cw antigens. Antibody strength was determined as strong, moderate or weak based on fluorescence channel shifts and x-median values determined based on intralaboratory comparison studies between antibody strength and the FCXM assays results. We cannot stress enough the need for each laboratory, in agreement with their transplant center, to determine their specific criteria for defining antibody strength. Notwithstanding this, the x-median values used by our laboratory are 10–50 for weak antibodies; 50–300 for moderate antibodies and >300 for strong antibodies (1024 channel, 4 decade log scale). Figure 1 provides an example of antibody strength analysis. Clearly, there is continuous gradation even within each strength level.

Patients on the waiting list were monitored once every 2–3 months for changes in their %PRA. Antibody identification testing was repeated on an annual basis. For these additional tests either the flow-PRA or luminex-based assays including the LabScreen (One Lambda.) or the Lifecodes (Tepnel Lifecodes Corp., Stamford, CT) kits were used. For this particular study, all patients had their most recent antibody identification testing performed on serum samples obtained up to 7 months prior to the date of the sample used for actual FCXM testing. In cases where discrepancies were observed between the actual FCXM and the vXM, antibody identification testing was performed on the same serum sample used for actual FCXM.

Virtual XM

To perform vXM, donor HLA antigens were compared against the potential recipient's HLA-antibody specificities (as described above). Whenever possible all donor HLA antigens were used for analysis, including HLA-A, -B, -Cw, -DR and -DQ. For the purpose

of vXM, all DSAs were considered, whether they were strong, moderate or weak. The results of both class I and class II XM were considered. The vXM was performed by an individual blinded to the actual FCXM results.

Results

Initial evaluation

Virtual XM results were assigned blindly, as described in the method section. The vXM results were then compared with actual FCXM data in a 2×2 table (Table 1). The vast majority of XM (1334/1480; 90.1%) were in concordance—480 XM (32.4%) were positive by both the actual FCXM as well as vXM. Similarly, 854 XM (57.7%) were negative by both XM methods. Sixty-three (= 4.3% of total XM) were positive by the actual FCXM but predicted to be negative by the vXM. Eighty-three (83 = 5.6% of total XM) were negative by the actual FCXM but predicted to be positive by the vXM. This translated into 85.3% sensitivity and 93.1% specificity of the vXM data to accurately predict actual FCXM results based on solid-phase-based antibody identification data. This, in turn, provides a positive predictive value of 88.4% and a negative predictive value of 91.1%; a positive likelihood ratio of 12.4 and a negative likelihood ratio of 0.16.

Supplementary information

These results were somewhat surprising, especially since based on our previous experience of analyzing data for recipients of living kidney transplantation we expected to find both a higher positive predictive value and a higher specificity. Upon reexamination, we found that 61 of 63 patients with discordant results (FCXM positive/vXM negative (4% of all XM) were never tested for the presence of antibodies against one or more of the donor HLA antigens. As mentioned above, cost constrictions mandated the testing for antibodies only against the more frequently encountered HLA antigens. HLA antigens that are less frequent in the population were not used at the time of initial analysis and patient listing for deceased donor organ. Some examples include antibodies to HLA-A74, HLA-A80, HLA-B42 or HLAB72. To enable the prediction of a more accurate vXM, we performed the required additional testing for all patients with a positive actual FCXM but a negative vXM (N = 63). In 35 of the 63 cases (56%), DSA—explaining the positive actual FCXM—were found. Interestingly, in 14 of the 35 patients (40%) the only, or at least the strongest, DSA explaining the actual FCXM result was directed against donor HLA-Cw antigen. Of note, 29 of the 63 patients (46%) that required additional antibody work-up had antibodies directed at HLA-Cw antigens.

The importance of obtaining a complete DSA profile: An example of the inability to complete vXM without additional analysis

A donor–recipient pair was tested as strong T- and B-cell positive actual FCXM while initial vXM was negative. As shown in Table 2, DSA information was not available for all donor HLA antigens and therefore the presence or absence of DSA could not have been ascertained, or ruled out. In this particular case, using the extended antibody panel, we found that no DSA were present against B42 or B50. However, strong antibodies to HLA-Cw6 and moderate antibodies to Cw17 were detected (see Table 2 supplemental information). These

findings explained the strong positive actual FCXM result observed and our initial assignment of what turned to be a 'false' negative vXM.

Comparison following the supplementary evaluation Actual FCXM positive; vXM negative

Once we had verified that each recipient had a complete relevant antibody profile with which to perform vXM, we repeated the assignment of the virtual assay. In this cycle, we increased the concordance between positive actual FCXM and positive vXM from 480 to 515 and reduced the number of a negative vXM in the face of a positive actual FCXM from 63 to 28 (Table 3). This translated into increased sensitivity—86.1% and more importantly increased specificity—96.8%. The positive predictive value was also significantly increased to 94.8%. Positive likelihood ratio was of 26.9 and negative likelihood ratio was of 0.14. We have further compared between actual FCXM and vXM grouping patients based on their degree of sensitization: patients with low levels of sensitization with PRA <20%; patients with moderate levels of sensitization with PRA between 20% and 80%; and highly sensitized patients with PRA >80%. Data are presented in Figure 2.

Of the remaining 28 discrepant assays, 21 were T-cell and B-cell positive and 7 were T-cell negative B-cell positive. Eleven of these patients had 0% PRA by solid-phase-based assays in repeat testing, using both flow cytometry and luminex tests. These actual FCXM-positive vXM-negative assays are likely due to non-HLA antibodies such as autoantibodies, or due to technical issues in performing the FCXM assay. We considered these assays as 'false' positive FCXM, that is, not due to HLA antibodies.

Actual FCXM negative; vXM positive

Eighty-three XM were predicted to be positive by vXM but the actual FCXM was negative. Forty-three (52%) of these patients presented with a single, weak, DSA as part of their antibody profile. A possible explanation for these actual FCXM negative vXM positive cases may be the increased sensitivity of the solid-phase testing employed for identifying DSA.

The remaining actual FCXM-negative vXM-positive patients (N = 40) presented with a single moderate DSA, yet the actual FCXM was negative. These cases were evaluated for the presence of DQA and DP antibodies and to a variety of HLA class I alleles. While we did not have reagents to investigate all class I allele-specific antibodies, at least in some cases the recipient had antibodies to a different allele of the antigen group to which the donor HLA allele belongs, but not to the allele that the donor expressed. Alternatively, the donor might have had different Alpha-Beta chain combinations—this applies mainly to antibodies against DQ antigens—that differed from the reagents used to perform the test. In one example the recipient HLA type was HLA-A2, A26; B44, B60; DR4, –; DQ8, –; and the donor HLA type was HLA-A2, A3; B7, B60; DR4, –; DQ7, DQ8. The vXM was calculated to be positive based on the presence of strong antibodies to the donor HLA-DQ7 (other HLA antibodies expressed by this patient were: strong antibodies to DR103, DR7, DR12; DQ2, DQ4; moderate antibodies to DR13 and weak antibodies to DR11, DR14, DR15; DQ5, DQ6). The projected vXM result was in disagreement with the actual FCXM results performed by the OPO laboratory, which were negative for both T- and B-cells.

The results of the ensuing extensive antibody evaluation are presented in Table 4. We performed further high-resolution typing of the donor HLA-DQ7 as DQA1*0301/DQB1*0301. Based on the additional information, it was clear that the recipient indeed had antibodies to some DQ7 alleles (Table 4) but had no antibodies to other DQ7 alleles, and specifically to the DQ7 allele expressed by the donor (Table 4). A transplant in this particular case would have been safe from the immunological perspective (should all that information have been available at the time of decision making).

Antibody strength

Table 5 summarizes the antibody strength composition in the concordant positive actual FCXM-positive vXM assays, in comparison with the negative actual FCXM-positive vXM assays. As expected, the proportion of strong DSA is significantly higher in the positive actual FCXM group (28.5% vs. 4.8%). The presence of weak DSA, and significantly the presence of only a single weak DSA, is considerably lower in the actual positive FCXM group (16.9% vs. 53.0% and 6.41% vs. 42.17%, respectively).

Table 6 presents the likelihood of having a positive actual FCXM based on the strength of the DSA. Strong DSA will translate into a positive FCXM in 97% of the cases; moderate DSA in 86% of cases and weak DSA in 66% of actual FCXM. When patients with weak DSA were divided into patients with multiple or single weak DSA, the translation to a positive FCXM was 86% and 49%, respectively.

Discussion

In this retrospective cohort blinded study we demonstrated that reliable results can be obtained using vXM with the caveat that a complete battery of reagents are used in the assessment of donor HLA antigens and a thorough analysis is employed when defining the true recipient antibody profile for DSA. Two potential observations can be problematic: the interpretation of results demonstrating a positive actual XM result in the presence of a negative vXM; and its converse situation—interpreting a negative actual XM result in the face of a positive vXM.

Positive actual XM with negative vXM

The implication of this observation is that some transplant candidates may be denied the chance of receiving a transplant despite the absence of donor-specific HLA antibodies. The results of our study clearly indicated that some of the discrepancies between actual FCXM and vXM results were due to incomplete evaluation of the recipients' antibody profile. We have shown that of the 63 cases that were initially included in this category, 61 did not have complete DSA analysis due to the presence of less frequent HLA antigens in some donors. This was found to be primarily due to the fact that our center was not routinely identifying antibodies directed against HLA-Cw antigens. While the clinical significance of such antibodies has yet to be firmly established, in 40% of the cases of positive actual FCXM with 'negative' vXM—the only or the strongest antibody to explain the actual FCXM was the antibody directed against donor HLA-Cw antigen. Indeed, although these results do not provide information about the potential clinical significance of HLA-Cw antibodies, they do

appear to indicate that recipient anti donor HLA-Cw antibodies may cause a positive FCXM (19–21).

Antibodies to HLA-Cw antigens are difficult to detect using serological methods, because of the lower cell surface expression level of these antigens. This may also contribute to their perceived lower immunogenicity. The availability of solid-phase based single-antigen assays provided the tools to detect and identify these antibodies. In fact, among the patients that underwent additional testing, antibodies to HLA-Cw specificities were detected in 48% of patients. Thus, the prevalence of such antibodies among sensitized patients seems to be fairly high and probably warrants a call for routine typing of donor HLA-Cw antigens as well as identification of recipient HLA-Cw antibodies.

The balance of discrepancies in this category (28 out of the 63 remaining after the additional work-up) is due, at least in part, to technical issues associated with the actual FCXM assay. Eleven of the 28 patients (39%) had no HLA antibodies as measured by multiple solid-phase-based assays and in multiple serum samples. While it is conventional to view XM results as a 'gold standard', it is crucial to remember that the XM assay is a biological assay. Even the more sensitive FCXM is not specific for the presence of HLA antibodies. A positive XM assay can be due to the presence of autoantibodies or other non-HLA-specific antibodies, an occurrence that should not be considered a contraindication to transplantation (22). Given this information, the negative vXM cannot be considered a false negative. In fact, it has been shown by us and others that transplantation in the face of a positive FCXM but in the absence of donor-specific antibodies does not increase the risk of early immunologic complications. It is imperative though to verify that if a patient does have HLA antibodies, even in the absence of DSA, that all donor-specificities (HLA-A, -B, -Cw, -DR, -DQ and potentially DP) have been investigated before concluding that the actual FCXM is false positive.

Negative actual XM with positive vXM

The implication of this observation is that some transplant candidates may receive a transplant and be unknowingly exposed to increased risk of long-term complications related to DSA because of less detailed pre-TX knowledge of potential immunologic complications. This is not to say that the presence of positive vXM should automatically be interpreted as contraindication to transplant. The strength of the antibody and additional clinical parameters (such as plausibility of performing protocol biopsies, etc.) should be considered prior to final decision whether to transplant or not. The example provided in Table 4 illustrates some of the limitations of the vXM, specifically in this case where the donor expresses different combinations of DQ α -DQ β chains compared with the reagents available for antibody identification. While it seems that high resolution typing of donor DQA and DQB alleles will be beneficial to resolve such discrepancies, and will definitely increase the accuracy of vXM, currently there is not enough information regarding the frequency of such allele-specific antibodies in the population. We advocate the initiation of a cost-benefit study to evaluate this issue as we enter the era of vXM.

Fifty-one percent of the actual FCXM-negative vXM-positive transplant candidates had only weak DSA and in 35 of the 83 cases (41%) only a single weak DSA was detected. The

implications of the clinical relevance of low level DSA at the time of transplant has been investigated by multiple groups (5,11,16,23–29). Fortunately, the presence of low levels DSA at the time of transplant is unlikely to result in hyperacute rejection (30), but it does signify the existence of a memory machinery (both T- and B-cells) as well as the presence of cells that currently generate antibodies against the donor. Some patients may be relatively susceptible to memory response activation (31–33). In those individuals, alteration of the immunosuppression approach may be attempted to modify DSA production. Other patients may have protective mechanisms that have not been thoroughly studied to date (34–37) although there is growing uncertainty regarding the role of these antibodies in long-term graft outcome (38,39). For example, regulatory T cells may be activated, potentially in a donor-specific manner that prevents activation of memory B and plasma cells. Additional explanation can be provided by the elegant work of Reed et al. (40–42) showing that low levels of HLA-specific antibodies may possibly lead to cell survival (as one of three potential consequences: proliferation, apoptosis or survival). When survival is the prevailing mechanism—antibody mediated rejection (AMR) may be avoided, hence translated to tolerogenic-like effects.

Currently, we do not have the appropriate tools and knowledge to distinguish protective from harmful antibodies. Monitoring DSA posttransplant, in those patients that have preformed DSA in the presence of a negative FCXM or a weak positive FCXM, would allow detecting variations in antibody levels posttransplant and may provide guidance for more beneficial, tailored, immunosuppression protocol.

The remainder 41 of the 83 (49%) patients with negative actual FCXM positive vXM were divided among a few patients with strong DSA (5/83; 6%) and patients with moderate DSA (36/83; 42%). Most of the patients with what was considered initially strong 'DSA', actually were found to have antibodies against different variants of that HLA specificity (see results). Among patients with moderate DSA, defining the strength of DSA remains challenging. While the solid-phase-based assays provide data regarding the level of fluorescence, they are not fully quantitative. Issues regarding the concentration of molecules attached to each bead surface; relationships between these numbers and cell surface expression on T- and B-cells (in general and in different individuals); variation in three-dimensional conformation, etc. affect our ability to consistently quantify the levels of antibodies. Indeed, this complicates the ability to compare data from multiple laboratories when weak antibodies are in question.

The final goal of our study was to determine an antibody strength threshold that is of clinical significance. Based on our results, the presence of strong, moderate or weak antibodies translated into 97%, 86% or 66% of the actual FCXM, respectively, given the cutoff used for this study. When weak DSA were divided into the presence of multiple weak DSA versus only a single weak DSA, it was evident that multiple weak DSA should be considered at least as an additive factor, whereas a single weak antibody was a poor indicator for a positive FCXM (49%). Given these results, we are currently working on redefining and optimizing the threshold for determining weak, moderate and strong antibody calls. We cannot stress enough the need for each laboratory, in agreement with their transplant center, to determine their specific criteria for defining antibody strength.

It is our view that each center has to determine their own threshold and cutoff policies, based on their current practices and level of immunological risk that is appropriate for their program (43). In fact, two center-specific guidelines should be determined:

Threshold 1—the actual level of antibodies in circulation at the time of transplant. What antibody level is a risk for hyperacute or accelerated AMR given a certain immunosuppression protocol? What level of antibodies can be removed in one cycle of PP/IVIg immediately pretransplant? What level of antibodies can be tolerated if the patient receives adjuvant Rituxan? What other factors play at hand (long-cold ischemia time, ECD donor, etc.)?

Threshold 2—level and function of memory and plasma cells—for long-term outcome—would dictate the need for some monitoring algorithm and potential changes in IS protocol. Can we develop tools to distinguish between mechanisms that will trigger accommodation versus those that will lead to slow rise in antibody secretion and long-term graft dysfunction?

In conclusion, vXM can serve as an outstanding tool to predict HLA compatibility between donor and recipient, with the caveat that the presence/absence of all antibodies against the potential donor, and their strength, have been thoroughly investigated.

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Figure 1. An example for HLA-antibody identification and strength assignment using a single antigen assay for flow cytometry

Nine different beads are presented in this example (multiplexed in one tube). The result field is divided into four fields. The leftmost field represents the negative control bead. The other three fields represent antibodies with weak, moderate and strong results against the individual beads. Each bead is coated with different HLA antigens, although the exact allele assignment for each bead is not presented here for clarity sake. Determination of the different strength categories is based on extensive work comparing antibody x-median values with FCXM results performed in our laboratory. These values should be generated by

each individual laboratory according to their specific practices and transplant guidelines. Current information suggests that converting information into MESF values will provide more accurate and more quantitative information. In this example there are four weak antibodies, three moderate antibodies and one strong antibody.

Figure 2. Actual FCXM and vXM results were compared in groups based on patients' degree of sensitization

Patients with low levels of sensitization with PRA <20%, light color; patients with moderate levels of sensitization with PRA between 20% and 80%, gray color; and highly sensitized patients with PRA >80%, dark color. Histograms represent percentage of each sensitization group within the four categories comparing actual FCXM and vXM. Figures in table represent the numbers of actual FCXM and vXM in each category.

Table 1

Comparison between actual FCXM results performed by the OPO laboratory with vXM results obtained by evaluating the presence of DSA to donor-specific HLA antigens; the initial analysis

	Virtual/DSA	
	Positive	Negative
Actual/FCXM		
Positive	480	63
Negative	83	854

Sensitivity = 85.3%; specificity = 93.1%; positive predictive value = 88.4%; negative predictive value = 91.1%.

Table 2

The ability to perform an accurate vXM is dependent on the availability of up-to-date information regarding the presence of antibodies against each and all of the donor HLA antigens

Recipient	A2	A-	B7	B35	Cw7	Cw-	DR1	DR-	DR52	DR53	DQ5	DQ-
Donor	A2	A3	B42	B50	Cw6	Cw17	DR4	DR18	DR52	DR53	DQ4	DQ-
DSA info	Shared	No DSA	No info	No info	No info	No info	No DSA	No DSA	No DSA	No DSA	No DSA	No DSA
Supplemental information			No	No	Strong	Moderate						
			DSA	DSA	DSA	DSA						

An incomplete information can result in an inaccurate vXM assessment.
Recipient and donor HLA typing is presented in the top two rows.
No information regarding the presence or absence of HLA antibodies to donor HLA-B42, B50, Cw6 and Cw17 was available at the time of the initial vXM. Supplemental work indicated the presence of strong and moderate DSA against Cw6 and Cw17, respectively, leading to the assessment of a positive vXM (as apposed to the initial negative result).

Table 3

Comparison between actual FCXM results performed by the OPO laboratory with vXM results obtained by evaluating the presence of DSA to donor-specific HLA antigens assuring that the presence of HLA antibodies against each and all donor HLA antigens have been assessed using solid-phase-based antibody evaluation

	Virtual/DSA	
	Positive	Negative
Actual/FCXM		
Positive	515	28
Negative	83	854

Sensitivity = 86.1%; specificity = 96.8%; positive predictive value; = 94.8%; negative predictive value = 91.1%.

Table 4

Evaluation of the presence/absence of different HLA-DQ7 variants based on its DQA/DQB chain combinations

Antigen ID	DQA	DQB	Strength	Luminex/flow
rDQ0301	0301	0301	Neg	L
G0124DQ0301	0601	0301	Neg	L
G0191DQ0301	0505	0301	Moderate	L
G0313DQ0301	0503	0301	Moderate	L
G0328DQ0301	0303/0505	0301	Moderate	L
C5036DQ7	0505	0301	Strong	F/L

The extended DQ luminex panel.

Neg = negative results, no antibodies detected.

Weak/moderate/strong = refers to the strength of the antibody.

L = luminex platform; F = flow cytometry platform.

Table 5

Antibody strength composition in concordant positive actual FCXM/positive vXM assays, in comparison with negative actual FCXM/positive vXM assays

	Total	%
FCXM positive/	515	100
DSA positive		
Strong DSA	147	28.5
Moderate DSA	279	54.2
Weak DSA	87	16.9
FCXM negative/	83	100
DSA positive		
Strong DSA	5	4.8
Moderate DSA	36	42.2
Weak DSA	44	53.0
Single weak	35	42.17%

Table 6

The likelihood of having a positive actual FCXM based on DSA strength

Antibody strength	Likelihood of positive FCXM
Strong DSA	97%
Moderate DSA	86%
Weak DSA	66%
Multiple weak DSA	86%
Single weak DSA	49%