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Review

Nothing's perfect: The art of defining HLA-specific antibodies

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ABSTRACT

The advent of solid phase assays and in particular the single antigen bead (SAB) assay, on the Luminex platform has led to previously unheralded levels of HLA-specific antibody characterisation. However, it soon became apparent that the detection of antibodies detected by these assays was less than perfect and that not all antibodies determined could be considered clinically relevant. Thus, the major challenges currently faced by HLA laboratories are to interpret the complex data provided by these assays and use this to devise a safe and practical algorithm for the definition of a clinically relevant HLA-specific antibody. Taking into consideration recent evidence and scientific opinion in this area we aim here to put forward the viewpoint of our laboratory in how best to manage the tricky problem of defining HLA-specific antibodies. By taking a balanced approach which is less reliant upon a single technique we propose that the aim should be to define antibodies to a level that does not discriminate against the highly sensitised patient, but also maintains clinical safety and efficacy. Knowing that not all of the antibodies detected by SAB are clinically relevant should lead to giving greater opportunity for patients with these antibodies having a crossmatch performed. In the future, more emphasis should be given to epitopes when interpreting the results of these assays.

Contents

1. Introduction

We like many others have struggled with the problem of the sensitivity of HLA antibody detection by Luminex and in particular the single antigen bead (SAB) technology. Many problems exist including variation in results, amount of antigen on the beads and whether antigens on beads have been denatured thus allowing recognition of cryptic epitopes [1]. Whereas some centres report association of donor specific antibodies (DSAs) with graft loss, others find the antibodies not clinically relevant [2–5]. Technical differences, especially MFI cut-off used, are one of the main contributors to these differences [6]. In addition the differences in the quantity of antigens on the beads, in particular augmentation of HLA-Cw, -DQ and -DP antigens lead to over estimation of antibody strength in a situation wherein there may be less immunological risk of rejection [7], although some centres normalise for the amount of antigen [8]. Zachary and colleagues have also reported that high negative or low positive control values may reduce the strength of HLA specific antibodies [9]. There are also situations when the antibody is deemed to be against a self-allele and individuals may also have antibodies against self DQB in combination with non-self DQA and vice versa [10]. In addition, it has been well documented that the SAB assay is prone to saturation, indicated by diluted sera giving the

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same MFI as those of undiluted sera, a result that can also occur because of interference by IgM or C1. Many laboratories now treat the sera with DTT or EDTA to remove interfering factors, which otherwise could lead to false low MFI levels.

Standardisation of reagents and protocols in seven laboratories has been shown to reduce variation in test results [11] and a recommendation that there must be quality control procedures to monitor inter- and intra-assay variability has been made by a group of experts [12]. In a previous study in four laboratories in the United Kingdom, testing the same six serum samples and using the same lot of SAB from the same manufacturer, although antibodies listed were in the same ranking order, there were differences in MFI values, in some cases as much as 250% [13]. This could lead to differences in what each laboratory took as unacceptable specificities, when the MFIs differed around the cut-off point.

There appears to be a drive to have all laboratories having tests standardised in order that results can be compared. Is this necessary? Surely it is more important for standardisation in a laboratory with the transplant programme of that unit. One exception to this is that if results are not reliable between laboratories, it can impact on kidney allocation. Because of this Eurotransplant does not consider antibodies defined by SAB for their acceptable antigen programme as this would dramatically affect the selection of the patients, many of whom could have clinically irrelevant antibodies [14].

2. Clinical relevance of SAB data

The listing of patients with incorrect unacceptable antigens was amply illustrated in a recent publication [15]. The difference in frequencies of antibody detection using SAB compared to CDC or enzyme-linked immunosorbent assay (ELISA) is striking, especially the differences between ELISA and SAB, as ELISA is thought to be the most sensitive technique after Luminex technology. Additional reports have shown that Luminex detected DSA twice as often as ELISA [16]. The presence of antibodies in so many patients with no known sensitisation, in particular some of these antibodies being against frequent alleles in the population, is particularly concerning [15]. Use of SAB kits from a second vendor did not clarify the data but instead raised additional queries. These results are similar to a previous report using SAB, which showed that 63% of 424 non-alloimmunised male blood donors had HLA antibodies [17]. However it also needs to be borne in mind that in a recent publication in patients positive by SAB but with unknown sensitisation, the flow crossmatch was positive in 47% of cases, indicating that because these antibodies are reacting with cell surface expressed antigens, they are not against denatured antigens [18].

Recent studies showed that new techniques, which allow the detection of antibodies, that fix complement led to many antibodies, which were positive in the initial SAB test, being negative [19]. Whereas some believe that antibodies positive with this new test are those with high MFI in the normal SAB test [20]; there is not universal agreement on this [21]. Others believe that the C1q test requires an enhancement from the manufacturer's recommended protocol [22].

Several studies have reported on successful antibody removal by desensitisation programmes. However some of these antibodies may have been clinically irrelevant. Could this be one reason why some desensitised patients are more likely to have antibody mediated rejection whereas others do not [23]? Obviously more work needs to be performed on this aspect. In this and other contexts of using SAB, it needs to be remembered that MFI levels do not represent titre. Indeed studies have shown that the correlation between MFI and antibody titre is reliable only within a very narrow range of concentrations and that this varies between individual beads within the assay [24].

3. The Liverpool experience

In this laboratory there are concerns on the issue of listing patients with unacceptable antibodies. In our defence we are continually thinking of ways to overcome the occasions when SAB may have detected non-clinically relevant antibodies. However, what happens in other laboratories? Are results of SAB assays challenged or is the tendency to list all antibodies above the threshold as unacceptable? How many centres follow SAB results over time, taking into consideration patient's history and querying the consistency of the test according to the operator, or positive control values? Do they remove unacceptable specificities if they are only present on one occasion or present in historical samples? It is not only testing with different vendor kits that leads to different results (vendors do not necessarily cover the same alleles and their source of antigen and associated peptide repertoire is different), but also testing the same serum with the same kit at different time points can lead to changes in unacceptable antigens determined. Using different vendors in the same laboratory, either at same time of testing or alternatively in the three month cycles of testing, may introduce more problems due to lack of consistency.

Despite these concerns, in a recent survey we conducted in UK laboratories, the overwhelming consensus was that there was a great deal of flexibility in each laboratory on how Luminex technology results were analysed, with much endeavour towards specific application to individual patients. The cut-off MFI ranged between 750 and 2000, but could change in some laboratories according to previous sensitisation or epitopes. All 12 laboratories who replied stated that on certain occasions they would ignore a DSA (even in the face of positive flow crossmatch), with two laboratories stating that they would be more concerned if these were against class 1 specificities. Five of the laboratories treated the sera with EDTA, although two only did this for SAB but not screening.

In this laboratory, electronic transfer of specificities with MFI greater than 1000 (the cut-off point used here) has been introduced from the Luminex software to the unit patient database to stop transcription errors, and work with one of the vendors has been undertaken to make the software more user-friendly. Thus the data for an individual patient can be interrogated more readily; for example, ascertaining if increasing the MFI cut-off for all samples of a patient would lead to a reduction in the number of unacceptable specificities. Cross-referencing the unacceptable specificities in the most recent sample with previous samples and ascertaining when the specificities in the current sample last appeared are also easily performed. Incorrect labelling of samples in the Dialysis Unit also requires awareness. Samples reportedly from one patient have, on careful analysis of SAB specificities, been shown to be from a different patient. Without the above scrutiny, the increased number of unacceptable antigens would exclude many patients from transplantation.

Unfortunately because of the algorithm for matching employed by UK Transplant and the degree of resolution generally employed in UK laboratories in typing deceased donors, it is not possible to list patients with allele specific antibodies or antibodies to HLA-DQA, -DPA and -DPB. In this laboratory whether a patient is listed as having the unacceptable allelic family (2-digit) when the antibody is only against a specific allele (4-digit) is dependent on the percentage frequency of the allele within the allelic family. An even greater problem arises when the patient has another allele of the same allelic family. In these instances it is not permissible to list the allelic family as an unacceptable specificity, even if so desired.

From experience some of the patients at the Liverpool Unit have unacceptable antibodies listed which in reality are not true antibodies. Knowing which antibodies are false is very difficult. In the live transplantation situation, the MFIs of donor specific antibodies (DSAs) have been compared with flow and complement dependent cytotoxicity (CDC) crossmatch results. MFI greater than 4000 or greater than 8000

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is roughly equivalent to a positive flow or CDC crossmatch, respectively. However, 1000 has continued to be the cut-off point, although this level is increased for patients for whom obtaining a kidney is difficult, due to the large number of unacceptable antigens.

Why has the policy not changed at this unit? The situation is somewhat analogous to previous decades in which it was easier to call a crossmatch positive when there was any doubt. So unacceptable antigens, when the MFI is greater than 1000, are listed to be sure rather than sorry. The association of prolonged cold ischaemic time (CIT) with delayed graft function, with possible effect on graft survival, has led to CIT being reduced as much as possible, especially for donors after cardiac death (DCD) [25]. This encourages the laboratory to list all possible unacceptable antigens for fear of a positive crossmatch, ultimately denying some patients the opportunity of having a crossmatch performed. In this laboratory, CDC and flow crossmatches are carried out for all patients who require a prospective crossmatch i.e. those for whom virtual crossmatch is not performed. If MFI greater than 1000 was used as a cut-off, the only problem that could arise is that the crossmatch might be positive. We now believe it is time for this scenario to be revisited and a calculated risk of a positive crossmatch to be taken for certain patients, who otherwise would not receive an offer. Patients may have high or low responding immune systems and some may be able to withstand moderate levels of DSA whereas others will not. In certain circumstances at this unit the transplant may still be performed in the face of a CDC negative, flow positive crossmatch, with a lot depending on the clinical situation of the patient. In practice an attempt is made to determine the risk criteria for each specific patient.

Recently a report indicated that a very small percentage of samples (3/534) had to be repeated because the positive control values were below 5000 MFI [15]. This intrigued us somewhat as in our laboratory 20% of samples had positive controls below 5000. After analysing this problem, it was concluded that the problem was in the washing technique. We have now introduced an automated vacuum washing technique which greatly reduces the inter-operator variability. However, resolving the problem not only resulted in positive controls always being above 5000 MFI but also led to more individuals having more antibodies detected with MFI greater than 1000. Some of these results were “odd”. Thus, the cut-off point for unacceptable antigens has been changed to an MFI of 2000 and a record of antibodies between 1000 and 2000 is kept, but not listed as unacceptables, to help in analysis of crossmatches.

4. Approaches to define unacceptable antigen mismatches

Much is made of equality in the selection process and many transplant organisations use defaulting of HLA antigens common in one ethnic population to its nearest equivalent in a different ethnic population. However, if all antibodies with reactivity greater than 1000 MFI are listed, including some probably not relevant, discrimination is against females who have had children.

At the very least we firmly believe from our own experience that the SAB test cannot be used in isolation from a Luminex screening test. Whereas there was good correlation between flow and cytotoxic crossmatches and Luminex panels the correlation with SAB was substantially lower [9]. It would have been of interest to know how many of the samples in the Gombos et al. publication positive by SAB were also positive by Luminex screening [15]. They have certainly highlighted the dangers of going straight to SAB without a pre-screen. In a single antigen bead if the antigen is denatured [it is known that different antigens vary in that capacity [26]], there will be complete non-specific binding, whereas in a panel of beads, such as those which comprise the Luminex screening assay, the denatured antigen may be only one of six antigens on the bead resulting in less non-specific binding [27]. Gebel and colleagues have reported a serum with no detectable HLA antibodies in screening but with HLA-C locus antibodies in SAB. These antibodies did not react with cell lines from which the HLA-C antigens were derived suggesting recognition of denatured antigen in the SAB assay [22]. One method to check if results are due to epitopes exposed on denatured beads is to denature the antigen on the bead by acid treatment and repeat the test. It is much easier to contemplate the results if the original results were solely due to denatured beads, but not if due to a mixture of denatured antigen and normal antigen. Like all aspects of working with the bead technology, this denaturing of antigen has vast cost implications, which is probably the single greatest hindrance to progress in the correct application of this technology to solid organ transplantation.

There are other indications that possible transplants should not be automatically ruled out on the basis of SAB data. The Barcelona group refers to a 67% graft survival in patients who had MFI > 3000 to DSA [28]. This is poor compared to 87% in patients with MFI less than 3000 to DSA, but should be more appropriately compared to a population of patients remaining on dialysis and at least these patients did have the opportunity for a transplant, although whether this is the best use of the organ needs to be borne in mind. One of us (DL) has specific experience in the HLA incompatible transplant setting where it was demonstrated that death censored 5 year graft survival of 80.4% in DSA+ve transplants is achievable. Although CDC+ve XM transplants did less well (65% graft survival at 5 years), those transplants that were CDC−ve/FC−ve/SAB+ve experienced decreased graft survival only when pre-transplant DSA levels exceeded 10,000 MFI [29]. The fact that pre-transplant DSA levels of up to 10,000 MFI led to outcomes comparable to those of DSA−ve transplants lends further support to the idea that the threshold MFI for listing unacceptables is often set too high. In retrospect, it is highly likely that some of these cases did not have clinically relevant DSA and that over-cautious interpretation of SAB data has led to inclusion in an antibody incompatible transplant programme.

The problem of listing false unacceptables is too easily dismissed by the fact that on many occasions these antibodies are against rare specificities in the population [30]. Recently each individual SAB was ranked by calculating the occasions it was positive on testing in this laboratory and dividing by the frequency in the population of the HLA antigen on the bead. Of 22 class I specificities with a ratio >10, ten were present in the population at frequencies of 2–3% (Tables 1 & 2). Therefore, accumulation of these frequencies could prevent patients receiving transplants needlessly. In addition, this does not allow for the detection of false antibodies against common specificities. Indeed in the North West England population where the Liverpool Transplant Unit is situated, HLA-B*08:01 (one of the antigens which is positive by SAB but negative by ELISA in the publication by Gombos et al. [15]) is present at 29.9% (www.allelefrequencies.net), compared to the smaller percentage (12.5%) quoted in the Gombos et al. publication [15].

Furthermore only six of 79 class I specificities were below one, whereas five of 15, five of seven specificities, and two of seventeen were below one for HLA-DR, -DQ and -DP respectively (Tables 1 & 2). For some specificities this may be explained by the sharing of epitopes between antigens, for example A25 and A32 antibody reactivities are observed far more often than would be expected from their population frequency, due to their expression of the Bw4 public epitope. However, 14 specificities, all class I, that had a ratio of >100 and were all present in the population at less than 1%, are much more difficult to explain. Some appear to lack the expression of common epitopes that would lead to this increased reaction frequency. This is not always the case; for example antibodies to the uncommon allele HLA-B*67:02 are found frequently, but this could be due to the sharing of an epitope between HLA-B*67:02 and HLA-B*07:02. In fact, analysis of the epitopes identified by El-Awar et al. [1] reveals that B*07:02 and B67:02 share at least eight defined epitopes to which anti-HLA reactivity has been demonstrated. Testing by CDC to prove or disprove the validity of these positive results on beads with these antigens would be difficult due to the low frequency of these antigens.

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Table 1
HLA class I antibody prevalence. Expressed as a ratio of antibody frequency/antigen frequency. Of 79 class I specificities only five have a prevalence ratio <1. It should be noted that the frequency of the population having an antigen is given at a 2 digit resolution and thus includes all alleles of that allele family, whereas each single antigen bead has only one four digit allele present. Thus, if anything, the prevalence ratio is underestimated. Ratios >100 are darkly shaded and those between 10 and 99 are lightly shaded.

HLA antigen	A1	A2	A3	A11	A23	A24	A25	A26	A29	A30	A31	A32	A33	A34	A36	A43	A66	A68	A69	A74	A80
Antigen frequency (%) n = 2131	35.85	50.02	24.21	13.28	3.52	14.17	3.24	3.47	6.95	5.35	5.26	5.63	2.02	0.28	0.14	0.00	0.47	8.07	0.05	0.19	0.09
Antibody frequency (%) n = 346	24.86	27.75	25.43	34.97	36.71	40.75	42.49	35.26	29.77	24.86	23.41	36.42	30.06	35.84	25.43	30.06	42.20	41.04	32.66	22.54	27.17
Antibody prevalence ratio	0.69	0.55	1.05	2.63	10.43	2.88	13.12	10.15	4.29	4.65	4.45	6.47	14.90	127.29	180.66	∞	89.92	5.08	695.96	120.10	289.47

HLA antigen	B7	B8	B13	B18	B27	B35	B37	B38	B39	B41	B42	B44	B45	B46	B47	B48	B49	B50	B51	B52	B53
Antigen frequency (%) n = 2131	26.14	29.19	3.47	7.13	7.88	12.53	2.86	1.74	4.46	1.17	0.33	29.05	1.50	0.33	1.13	0.09	2.53	1.83	6.24	1.22	1.08
Antibody frequency (%) n = 346	24.57	27.17	36.13	25.43	39.88	28.61	36.71	31.79	24.28	30.06	26.88	41.04	40.75	26.30	32.66	26.88	40.17	33.82	36.99	36.42	33.24
Antibody prevalence ratio	0.94	0.93	10.40	3.57	5.06	2.28	12.82	18.31	5.45	25.62	81.83	1.41	27.14	80.07	29.00	286.39	15.85	18.48	5.93	29.85	30.79

HLA antigen	B54	B55	B56	B57	B58	B59	B60	B61	B62	B63	B64	B65	B67	B71	B72	B73	B75	B76	B77	B78	B81	B82
Antigen frequency (%) n = 2131	0.05	3.52	0.52	6.43	1.36	0.00	10.75	1.17	11.22	0.75	2.02	4.74	0.05	0.56	0.09	0.05	0.47	0.05	0.05	0.00	0.05	0.05
Antibody frequency (%) n = 346	28.03	29.77	28.90	39.88	35.84	33.24	32.95	32.08	30.64	39.60	17.92	18.79	30.92	27.17	27.75	27.75	38.73	47.40	34.39	28.61	26.01	38.44
Antibody prevalence ratio	597.42	8.46	55.99	6.20	26.33	∞	3.07	27.35	2.73	52.74	8.88	3.96	659.01	48.25	295.63	591.26	82.53	1010.07	732.92	∞	90.00	133.00

HLA antigen	Cw1	Cw2	Cw4	Cw5	Cw6	Cw7	Cw8	Cw9	Cw10	Cw12	Cw14	Cw15	Cw16	Cw17	Cw18
Antigen frequency (%) n = 1706	6.57	6.15	16.30	21.98	16.88	66.24	8.56	9.09	15.83	7.15	1.35	4.04	6.68	1.47	0.06
Antibody frequency (%) n = 346	17.63	24.86	16.47	17.63	19.94	8.67	10.98	18.79	19.65	13.01	13.29	23.70	12.14	28.03	19.08
Antibody prevalence ratio	2.69	4.04	1.01	0.80	1.18	0.13	1.28	2.07	1.24	1.82	9.86	5.86	1.82	19.13	325.42

A contemporary publication showing algorithms of how five European and one South American laboratories attempt to deal with the problem, sheds some light [28]. Therein, it becomes apparent that screening by complement-dependent cytotoxicity (CDC) still has a role to play, despite the method not detecting all clinically harmful antibodies. This is also apparent from the Gombos et al. publication [15] and from the recent recommendations from a panel of experts that CDC screening should be applied [12]. It has become apparent that as a community we have been quick to dismiss CDC screening, no more so than in this laboratory. Many in the HLA community believes in crossmatching by CDC, mostly augmented with flow crossmatch, despite limitations in sensitivity and specificity. However, screening by CDC was a cumbersome, inconvenient method that certainly this laboratory was glad to remove from its repertoire. Now, we need to revisit the problem. One innovative idea of colleagues at Eurotransplant was the development of a panel of single HLA antigen-expressing cell lines [14]. We believe that we cannot use the Luminex technology as a stand-alone test. As recommended, this laboratory is re-introducing a cell-based assay. Results from CDC screening and SAB will be analysed according to epitopes and previous sensitisation events to ascertain the degree of risk. Thus the results from the Luminex technology can be considered as a risk factor, below that of a positive CDC crossmatch and a positive flow crossmatch, but not necessarily a preventative factor. Interestingly only two of the 12 laboratories in the UK survey previously mentioned, used, in addition to SAB, another method (CDC) to detect unacceptable antigens.

We are not advocating for one minute not using the SAB technology. It is too easy to forget how laboratories used to test for antibodies. But remember in the bad old days of simply screening by CDC to select sera for a CDC only crossmatch, graft survival rates at five years of 65% were obtained in the era 1968–78 long before modern immunosuppressive agents were available [31]. The SAB technology has allowed the introduction of virtual crossmatching with the transplant going ahead without waiting for a crossmatch result, thus decreasing ischaemic times. (This does require the continual receipt of serum samples every three months and knowledge of current sensitisation events, facts not always appreciated by the Dialysis Unit, although in the four years of operating this system a positive retrospective crossmatch has never occurred.) Indeed in this unit, when a DCD donor is received, initially a patient on the virtual list is transplanted (this includes patients with fixed and stable antibodies when tested by SAB every three months) at the same time performing the crossmatch on possible recipients with antibodies for the second transplant. (In the UK a centre is allowed to keep both kidneys from a DCD donor.) We, like others, see this as one of the main benefits of SAB technology [32]. However because of the small pool of awaiting recipients at our centre ($n = 400$) at any one time, using both DCD donors in one centre leads to very poor matching. Thus although CIT is kept to a minimum, the level of mismatching is concerning both to the future of this transplant and to sensitisation for any subsequent transplant.

Other benefits of SAB technology are readily apparent. A significant increase in the allocation of organs to highly sensitised patients (cPRA >80%) in the USA, has been deemed to be due to greater knowledge of the unacceptable specificities in these sensitised patients [33]. The technology also helps the interpretation of the lymphocyte crossmatch, especially those positive crossmatches not due to HLA antibodies and in those patients who are receiving drugs that interfere with the crossmatch. Indeed in some disputable crossmatches the SAB may be negative and the transplant may take place [34].

There are occasions when the true breadth and level of antibody reactivity can be underestimated and it is also necessary not to automatically dismiss these low level MFI HLA-specific antibodies. Could these be a sign of prior sensitisation and what risk factor do they impose? Again because of cost it is not reasonable and justifiable to perform SAB testing more often than once per year leading to the danger of missing temporal changes in antibody content. Another problem to look out for is the spreading of an antibody (e.g. HLA-Bw4) in which the antigen is present on many beads leading to MFI on each of the beads being below the positive threshold. One way to attempt to counteract this problem is to use single beads with only one specificity bead in the test possibly, if known, using the original sensitising antigen.

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Table 2
HLA class II antibody prevalence. Expressed as a ratio of antibody frequency/antigen frequency. Ratios >100 are darkly shaded and those between 10 and 99 are lightly shaded.

HLA antigen	DR1	DR4	DR7	DR8	DR9	DR10	DR11	DR12	DR13	DR14	DR15	DR16	DR17	DR18	DR103
Antigen frequency (%) n = 2131	18.91	38.24	21.73	4.36	2.02	1.74	9.57	3.19	16.14	2.91	23.28	0.99	30.69	0.23	4.13
Antibody frequency (%) n = 346	15.90	20.52	20.23	19.65	21.97	15.61	24.28	23.41	23.12	19.65	15.90	17.34	18.79	17.34	18.79
Antibody prevalence ratio	0.84	0.54	0.93	4.50	10.89	8.99	2.54	7.34	1.43	6.75	0.68	17.60	0.61	73.91	4.55

HLA antigen	DQ2	DQ4	DQ5	DQ6	DQ7	DQ8	DQ9
Antigen frequency (%) n = 2019	45.81	4.21	27.59	37.89	30.21	23.18	8.22
Antibody frequency (%) n = 346	16.18	21.97	21.10	22.83	24.86	22.54	24.86
Antibody prevalence ratio	0.35	5.22	0.76	0.60	0.82	0.97	3.02

HLA antigen	DP1	DP2	DP3	DP4	DP5	DP6	DP9	DP10	DP11	DP13	DP14	DP15	DP17	DP18	DP19	DP23	DP28
Antigen frequency (%) n = 128	14.84	21.88	16.41	87.50	3.13	8.59	0.78	2.34	3.13	6.25	1.56	0.78	3.13	0.78	0.78	0.00	0.00
Antibody frequency (%) n = 346	19.36	15.03	17.34	13.58	13.29	11.56	15.61	16.47	13.01	13.29	15.61	9.25	16.76	15.90	15.03	9.54	14.74
Antibody prevalence ratio	1.30	0.69	1.06	0.16	4.25	1.35	19.98	7.03	4.16	2.13	9.99	11.84	5.36	20.35	19.24	∞	∞

However, this is very costly and it appears the vendors are going to stop availability of supply of single SAB. Additionally, greater flexibility by the vendors in how many different beads are in the same batch would be helpful.

5. The use of epitope analysis to clarify SAB data

Future endeavours should concentrate on epitopes to ascertain if sharing of epitopes on different specificities could lead to a more meaningful conclusion on the validity of the test results. For example a recent serum sample tested in this laboratory showed a previously unsensitised patient reactive solely with the A*34:01 bead on class I SAB. Further analysis showed that A*34:01 differed from the A*34:02 protein also present on the panel at four distinct amino acid positions. Crucially, none of these amino acid substitutions were exclusive to A*34:01 and were present on a number of other proteins found in the SAB panel. This data is easily analysed and can be used routinely in the laboratory to aid in the definition of true anti-HLA reactivity. The vendors should be encouraged to show epitope diversity in their products, although laboratories can assist themselves by using the Matchmaker concept [35]. It would not appear to have been their intention but Gombos et al. support this notion in their review with the observation that SAB reaction breadth is limited to far fewer beads in the patients who are positive by SAB only, compared to those who are also positive by CDC and ELISA [15]. The very nature of epitope sharing suggests that true HLA-reactive patients would react with greater numbers of beads within the SAB assay. In the list provided by Gombos et al. [15] that shows those specificities with the highest SAB reactivity it is interesting to note that of the three specificities that have the highest prevalence, two, HLA-B*37:01 and C*17:01, have no epitopes unique to that allele and the third, HLA-B*15:12 has only one according to the epitope list previously published by Terasaki et al. [26]. It would be

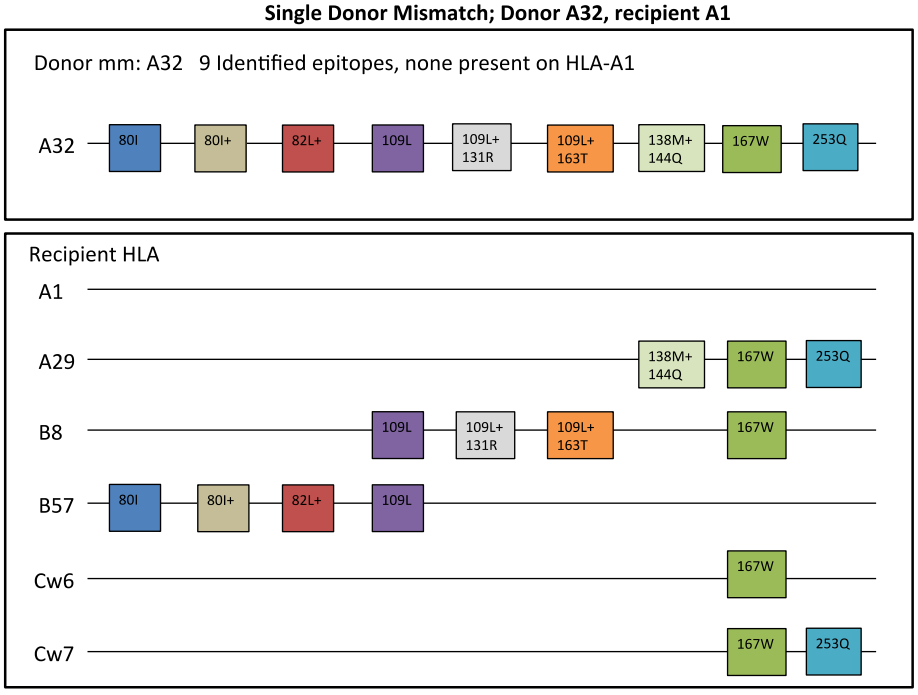

Fig. 1. The effect of matching for epitope on the degree of compatibility. The single class I mismatch, donor A32 for recipient A1 revealed nine potential mismatched epitopes. When the epitope distribution on the recipients of other HLA antigens was analysed it revealed that no epitope mismatches were present on the donor A32. Epitope designations according to El-Awar et al. [1].

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interesting to know if the samples containing these antibodies had other detected antibody specificities.

How to report and monitor DSA is another area of contention. Whereas some centres will sum MFI of mismatched antigens, others take only the highest. It seems more logical to sum if an epitope is shared amongst the donor mismatches. Part of the problem with epitopes is that the definition of epitopes recognised by antibodies is limited. Most epitope definition is based on structural differences between HLA molecules. Added to this are the differences in immunogenicity of the epitopes [36]. Perhaps thinking along these lines will lead to "epitope matching", whereby epitopes on mismatched antigens could be analysed to ascertain if these epitopes are present on other antigens of the recipient. With knowledge of the epitopes that are unacceptable it is realistic that the number of epitopes mismatched could be vastly reduced. This is illustrated in Fig. 1 which shows that although there is one mismatch (HLA-A32), in reality when all the HLA antigens of the recipient are shown there are no epitope mismatches.

Another ploy is to use "tracker" antibodies; i.e. following the epitope reactions and not the antigen reactions [37]. To track epitope-specific antibodies, beads are selected that carry the minimum number of each of the epitopes of donor specific reactivity, but on non-donor antigens. This approach has been shown to reveal that multiple antibodies specific for different epitopes on the same broad antigen can display unique kinetics in the early post-transplant period. Vendors should be encouraged to design SAB panels to reflect adequate coverage of epitopes rather than antigens. A further problem requiring addressing by the vendors is that many 2-digit allele families are represented on the SABs by only one 4-digit allele, which may not be the most common allele of that allele family in that population.

6. Summary

In summary, we believe that Luminex technology has led to certain improvements in transplantation. In particular, the ability to perform transplantation without crossmatching, in allocation to patients with antibodies, especially in heart transplantation, and in the detection of donor directed antibodies post-transplant. But now it is a prerequisite for all of us to look again. The two recent publications [15,28] should be a stimulus for that. What we and others suggest is that the results of SAB should be taken into consideration, but as a potential risk factor, to be considered along with the crossmatch result (a positive CDC on current serum is always considered a contraindication to transplantation) and clinical considerations of the recipient, including risk of delaying transplantation and likelihood of identifying a suitable alternative donor within a clinically accepted time frame, and to guide the selection of immunosuppressive agents, not to rule out the transplant without forethought.

Most things that are good for you have drawbacks, and as Gebel and Bray point out, the perfect scenario is not available [30]. But why dismiss the problem because of this? Moderation needs to be applied. Wine can be good for you in small quantities, but as many of us know from experience, abundance can lead to problems. So let's work together to ascertain how best to apply and use the new technology. If SAB antibodies can be differentiated into those harmful and not harmful, let's find a method to do so.

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