



Antibody monitoring system to support the single-antigen Luminex assay in donor-specific antibody detection

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ABSTRACT

The antibody monitoring system (AMS) is a crossmatch test using enzyme-linked immunoassay to detect donor-specific anti-HLA antibodies (DSA). The aim of this study was to determine whether the AMS assay is useful for supporting DSA results measured with single-antigen Luminex assay (Luminex-DSA) in renal transplant recipients. Thirty sera from 12 sensitized recipients and 71 sera from 60 nonsensitized recipients were screened by complement-dependent cytotoxic crossmatch (CDC-XM), flow cytometric crossmatch (FCXM), AMS, and the Luminex assays. Twenty-two sera (73.3%) from sensitized patients were positive for Luminex-DSA, and the AMS assay revealed the great specificity and positive predictive value for detecting Luminex-DSA in sera from sensitized patients. Positivity in the AMS assay was observed most frequently in Luminex-DSA-positive sera with $\geq 10,000$ mean fluorescence intensity (MFI) levels (66.7%). Of the 101 sera, the results of the AMS assay were compatible with CDC-XM results in 73 sera (91.3%) and with FCXM results in 72 sera (90.0%); these rates were significantly higher for the AMS assay than for the Luminex assay ($p = 0.008$ for CDC-XM and $p = 0.001$ for FCXM). In conclusion, the AMS assay is useful as a supportive solid-phase method for predicting actual crossmatch and high level of Luminex-DSA in sensitized patients.

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1. Introduction

Identification of donor-specific anti-HLA IgG antibody (DSA) is important in renal transplantation. The presence of DSA in pre-transplant recipients may cause antibody-mediated rejection, which leads to high rates of graft loss, and is predictive of chronic rejection and allograft failure [1–3]. The number of available test options for detecting DSA has increased markedly in the renal transplantation field in the past few decades.

Strategies to increase the sensitivity of the crossmatch tests include the use of the anti-human globulin-augmented complement-dependent cytotoxicity crossmatch (CDC-XM) and flow cytometry crossmatch (FCXM) assays [4,5]. Both tests increase the diagnostic yield in pretransplant immune monitoring, and they remain important in clinical decision making about transplantation and desensitization. However, these assays have some limitations because they do not distinguish between clinically relevant antibodies to the donor HLA and irrelevant antibodies directed against other cell surface antigen (Ag) [6,7].

More recently developed solid-phase assays allow detailed characterization of HLA antibodies in transplant candidates. The single-Ag Luminex assay has emerged as an ideal tool for detecting

DSA because it has high specificity and sensitivity. New testing techniques allow the quantification of DSA to a degree not previously possible using cell-based crossmatch tests [8]. However, several concerns have been raised, because the routine Luminex assay is too sensitive and may exclude potentially acceptable donor–recipient pairs, and the weak HLA antibodies detected in the Luminex assay are clinically irrelevant. In addition, the results of the Luminex assay are incompatible with those of cell-based tests in some situations [9–11].

The antibody monitoring system (AMS) is a crossmatch test using enzyme-linked immunoassay method with soluble donor HLA Ag. We have reported previously that the AMS assay produces similar results to the FCXM assay in renal transplant recipients and that it is useful as a solid-phase crossmatch test [12,13]. The aim of this study was to investigate whether the AMS assay is a useful method for supporting the single-Ag Luminex assay for DSA detection in renal transplant recipients. We also compared the ability of the AMS and Luminex assays to predict the CDC-XM and FCXM results.

2. Subjects and methods

2.1. Patients and serum samples

This study was approved by the institutional review board of Seoul St. Mary's Hospital (KC11RISI0743). A total of 101 sera were obtained from 72 patients between January 2010 and May 2011. All

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sera were screened for the presence of alloantibody by four tests: AMS, single-Ag Luminex, CDC-XM, and FCXM assays. Lymphocytes and HLA glycoproteins for the crossmatch test and AMS assay were prepared from each paired donor.

Figure 1 illustrates the number of serum samples depending on the type of renal transplantation and time of sample collection. Twelve patients were sensitized to HLA Ag and underwent the desensitization protocol for renal transplantation. A total of 30 sera were prepared from these patients, and 17 sera were obtained after use of desensitization protocol. Of these, 3 patients did not receive the renal transplantation. Thirteen sera were prepared from 10 recipients receiving ABO-incompatible transplantation, and 4 sera were obtained after use of anti-ABO antibody removal protocol. Therefore, 21 sera from these patients were obtained after use of anti-CD20 monoclonal antibody for desensitization or anti-ABO antibody removal and they were excluded when the AMS and Luminex assay were compared with crossmatch test. Routine renal transplantation was performed in 42 recipients and eight transplant candidates who were not transplanted were included in this study.

2.2. Immunosuppression and clinical data

The routine immunosuppressant for renal transplantation was cyclosporine A or tacrolimus (FK506) in combination with mycophenolate mofetil and prednisolone. Basiliximab was used as an additional induction therapy. The desensitization and anti-ABO antibody removal protocol was used as previously described [14,15].

The transplant patients were grouped by AMS or Luminex assay results before desensitization or anti-ABO antibody removal. The acute rejection was diagnosed by core needle biopsy and identified

into the Banff 1997 working classification of renal allograft pathology [16,17]. The estimated glomerular filtration rate (eGFR) was calculated using the abbreviated Modification of Diet in Renal Disease (MDRD) Equation [18].

2.3. AMS assay

The AMS assay (GTI., Waukesha, WI) was performed according to the manufacturer's instructions. Donor lymphocytes were solubilized with a nonionic detergent to prepare HLA glycoprotein. Aliquots of sera were stored at -80°C before analysis. The solubilized HLA glycoprotein was added to microwells onto which murine monoclonal antibody specific for HLA class I or class II had been immobilized. After washing the microwells containing the bound glycoproteins, the recipients' sera were added to the wells so that the antibodies in the sera attached to the donor HLA glycoprotein in the microwells. Purified goat anti-human IgG antibody conjugated to alkaline phosphatase was added, and the presence of bound antibodies was detected with *p*-nitrophenyl phosphate substrate. The control sera were provided by manufacture and all AMS assays included a negative, positive, and lysate control to assess the amount of HLA class I and HLA class II antigens captured from the lysate. The optical density was read at 490 nm on a BEP-III processor (Dade Behring, Marburg, Germany). Reactions were considered positive if the optical density was more than twice the average of the negative controls.

2.4. Single-Ag Luminex assay and DSA measured with the single-Ag Luminex assay (Luminex-DSA)

The single-Ag Luminex assay was performed according to the manufacturer's instructions using Lifecodes LifeScreen Deluxe kits (Tepnel Lifecodes, Stamford, CT). The microbeads coated with purified HLA class I/class II glycoproteins were incubated with $12.5\ \mu\text{l}$ of serum in 96-well plates for 30 minutes. After three washes with a vacuum manifold, the beads were incubated with $50\ \mu\text{l}$ of 1:10 diluted R-phycoerythrin (PE)-conjugated goat anti-human IgG for 30 minutes. After washing, the test samples were analyzed using the Quick-Type Version 2.4 User's Manual RUO program in LAB-Scan100 flow cytometer (Luminex, Austin, TX); both positive and negative controls were included. The positive criterion was a mean fluorescence intensity (MFI) >1000 .

In all patients and donors, HLA typing was performed by the DNA molecular typing method using reverse sequence-specific oligonucleotide probes using the RELI TM SSO HLA-A, B, DR Typing Kit (Dynal Biotech, Bromborough, UK). If the detected anti-HLA antibody measured by the single-Ag Luminex assay corresponded to the mismatched donor HLA Ag, we defined the detected anti-HLA antibody as positive Luminex-DSA. If two or more than Luminex-DSA were detected in one serum, the peak MFI level was defined as that of Luminex-DSA with strongest reactivity. The peak MFI level was used to classify the sera into weak ($1000 < \text{MFI} < 5000$), moderate ($5000 \leq \text{MFI} < 10,000$), or strong ($\text{MFI} \geq 10,000$) MFI.

2.5. CDC-XM test

Donor B cells were isolated using CD19 monoclonal antibody attached to beads and T cells were isolated by negative selection. A $1\text{-}\mu\text{l}$ quantity of donor cell suspension (2×10^6 cells/ml) was incubated with $1\ \mu\text{l}$ of recipient serum for 30 minutes at room temperature, $1\ \mu\text{l}$ of anti-human globulin was added, and the mixture was incubated for 30 minutes at 37°C . The cells were washed, rabbit complement was added, and the mixture was incubated for 60 minutes at room temperature. The cells were stained with acridine orange and ethidium bromide, and observed for cytotoxicity using an immunofluorescent microscope. The CDC-XM results for T and B cells were considered positive when cell death exceeded that of the negative control well by 20%.

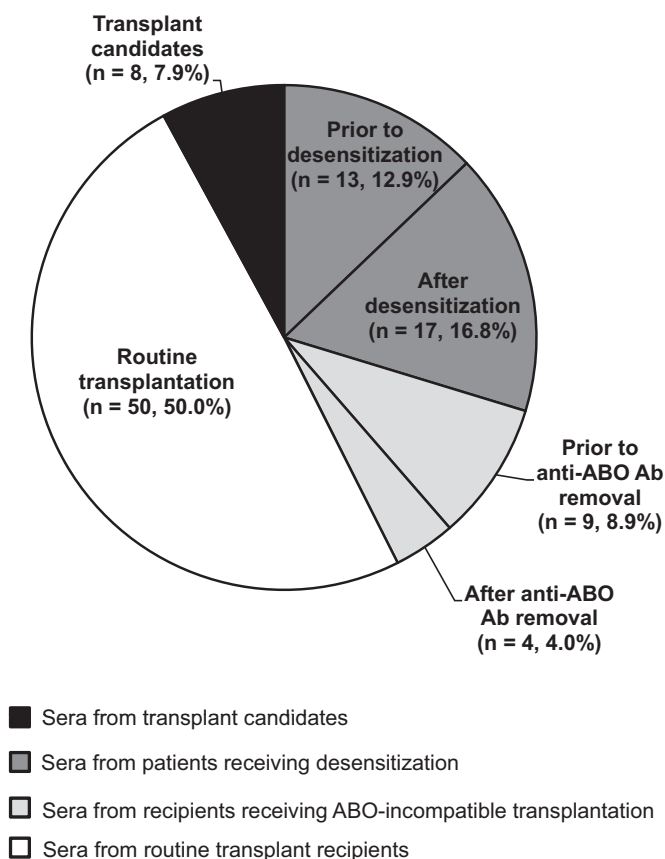


Fig. 1. Number of serum samples depending on the type of renal transplantation and time of sample collection. The relevant number and percent of sera were given in parentheses.

2.6. FCXM test

For the FCXM test, 2×10^5 donor lymphocytes were added to 50 μ l of sera and then incubated for 30 minutes at room temperature. Fluorescein isothiocyanate-labeled anti-human IgG (Dako, Kyoto, Japan) and PE-labeled CD19 or CD3 (Dako) were added, and the cells were incubated for 30 minutes. The cells were washed and then analyzed in a Coulter EPICS XL flow cytometer (Beckman Coulter, San Diego, CA). A positive FCXM test result was defined as a displacement of the mean channel fluorescence (MCF) by >10 channels relative to the negative control and donor autologous control. We also defined positive cases as a median relative fluorescence, calculated as $[\text{test MCF}/(\text{recipient autologous MCF} \pm \text{donor autologous MCF} \pm \text{healthy autologous MCF})/3] \geq 1.5$, and if the MCF was >3 SD above the negative control MCF.

2.7. Statistical analysis

The results are expressed as mean $\pm$ SD for continuous variables. Comparisons were made using the χ^2 test for categorical data. The degree of concordance between two tests is presented as the κ value, which was calculated from Kendall's tau-b test. To quantify the usefulness of the Luminex-DSA MFI as a predictor of AMS positivity, receiver operating characteristic (ROC) curves were plotted. Statistical analysis was performed using SPSS software version 15.0 (SPSS, Inc., Chicago, IL).

3. Results

3.1. Patients, anti-HLA antibody and Luminex-DSA status

The mean age of 72 patients was 42.4 ± 11.7 years, and 48 patients (66.7%) were men. The mean HLA mismatch number to paired donor was 3.5 ± 1.4 , and 10 patients (13.9%) had sensitization history of previous renal transplantation. There were no recipients receiving deceased donor transplantation.

The distribution of anti-HLA antibody detected by the single-Ag Luminex assay in 101 sera is illustrated in Fig. 2. The most prevalent anti-HLA antibodies were directed against HLA-DR Ag (30.7%). The prevalence of anti-HLA-C and anti-HLA-DQ antibody was rarely present, but anti-HLA-DQ antibody was detected in 23 sera (22.8%). All tested sera were evaluated for the presence of Luminex-DSA. A total of 72 sera (71.3%) were negative for Luminex-DSA HLA class I and II (Table 1). The positive Luminex-DSA were directed against HLA class I Ag in six sera (5.9%), against HLA class II Ag in 17 sera (16.8%), and against both class I and class II Ag in six sera (5.9%).

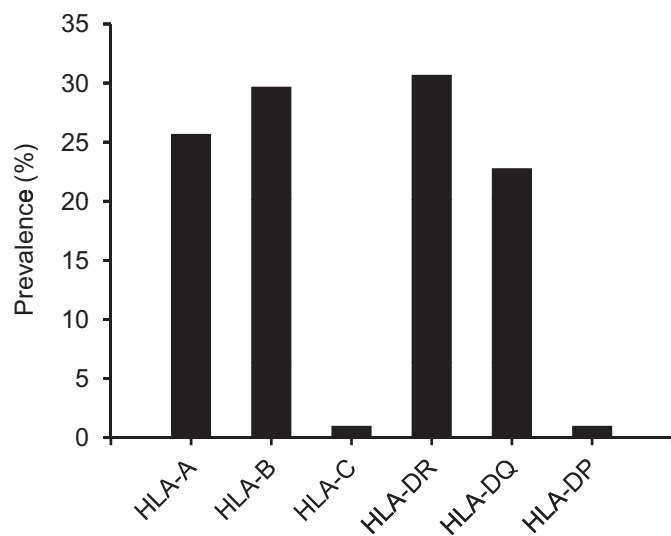


Fig. 2. Prevalence of anti-HLA antibody in studied sera.

Table 1

Comparison of the AMS assay with DSA detected by single-antigen Luminex assay

Luminex DSA	AMS assay			
	I–, II–	I+, II–	I–, II+	I+, II+
I–, II–	70 (69.3)	0	1 (1.0)	1 (1.0)
I+, II–	5 (5.0)	1 (1.0)	0	0
I–, II+	10 (9.9)	0	7 (6.9)	0
I+, II+	13 (12.9)	1 (1.0)	1 (1.0)	1 (1.0)

DSA, donor-specific antibody; AMS, antibody monitoring system; I, anti- HLA class I antibody; II, anti-HLA class II antibody.

3.2. Comparison between AMS assay and Luminex-DSA

The AMS assay was negative against HLA class I and II Ags in 88 sera (87.1%). In the AMS assay, two sera were positive for HLA class I Ag and 9 sera were positive for HLA class II Ag. Two sera (2.0%) were positive for both class I and class II Ags in the AMS assay. Of the 101 sera, the AMS results were compatible with the Luminex-DSA in 79 sera (78.2%). We evaluated the diagnostic accuracy of the Luminex-DSA MFI using a ROC curve to predict AMS positivity (Fig. 3). The area under the curve was 0.861 (95% confidence interval [95% CI] = 0.735–0.986, $p < 0.001$). The best cutoff threshold of the Luminex-DSA MFI for AMS positivity was 1945 (sensitivity, 84.6%; specificity, 85.2%).

Of the 71 sera from nonsensitized recipients, weak, and moderate Luminex-DSA was detected in six sera (8.5%) and one serum (1.4%), respectively. The AMS results were concordant with the Luminex-DSA in 63 sera (88.7%). Positivity in the AMS assay was observed in one serum with weak Luminex-DSA positivity and in two sera with Luminex-DSA negativity. The sensitivity of the AMS assay for the detection of Luminex-DSA was 14.3%; the specificity was 96.9%; the positive predictive value was 33.3%, and the negative predictive value was 91.2%.

Of the 30 sera from sensitized recipients, 22 sera (73.3%) were positive for Luminex-DSA. Nine sera (30.0%) had strong Luminex-DSA positivity, 4 (13.3%) had weak Luminex-DSA positivity, and 9 (30.0%) had moderate Luminex-DSA positivity. The AMS results were concordant with the Luminex-DSA in 16 sera (53.3%). Positiv-

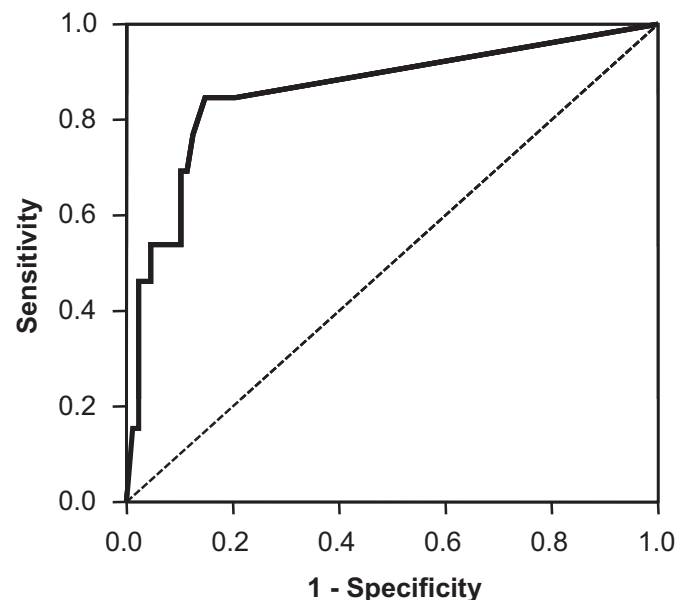


Fig. 3. Receiver-operating characteristic (ROC) curve to predict antibody monitoring system (AMS) positivity for donor-specific anti-HLA immunoglobulin G antibody (DSA) mean fluorescence intensity (MFI) of single-antigen Luminex assay in 101 sera. Area under the ROC curve was 0.861 ($p < 0.001$). DSA, donor-specific antibody; MFI, mean fluorescence intensity.

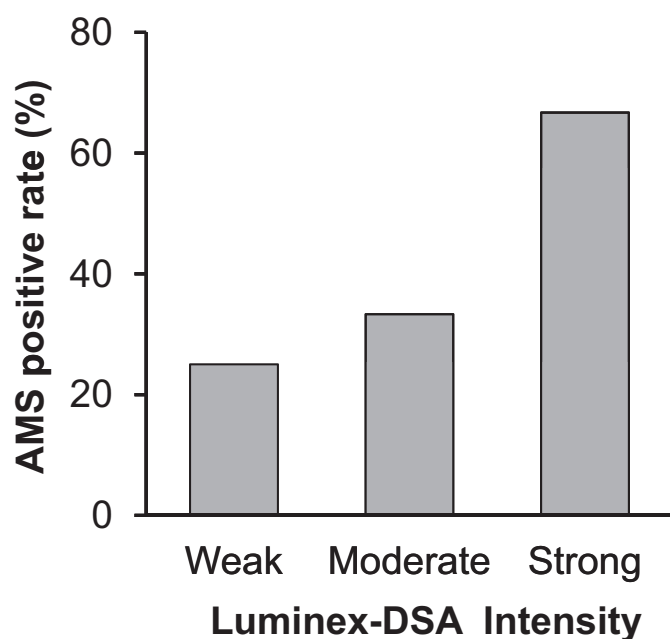


Fig. 4. Positive rate of AMS assay classified by Luminex-DSA intensity in 30 sera from sensitized recipients. The positivity for AMS assay was 25%, 33.3%, and 66.7% in weak, moderate, and strong Luminex-DSA-positive sera, respectively. MFI, mean fluorescence intensity.

ity in the AMS assay was observed most frequently in Luminex-DSA-positive sera with a strong MFI level (66.7%). In weak and moderate Luminex-DSA-positive sera, the positive rates for the AMS assay were 25% and 33.3%, respectively (Fig. 4). The sensitivity of the AMS assay for the detection of Luminex-DSA was 45.5%; the specificity was 100.0%; the positive predictive value was 100.0%, and the negative predictive value was 40.0%.

3.3. Comparison between AMS and Luminex-DSA with the CDC-XM assay

The AMS and Luminex assays were compared with the CDC-XM assay (Table 2). Of the 101 sera, 21 sera were excluded because rituximab or the desensitization protocol might have influenced the cell-based crossmatch tests. The AMS assay results were similar to the CDC-XM results in 73 sera (91.3%), and there was a significant degree of concordance ($\kappa = 0.54$, $p < 0.001$). Of nine sera with AMS positivity, 2 sera were positive for both HLA class I and class II Ags in the AMS assay. The CDC-XM was simultaneously positive for both T- and B-cell in one serum, but the other one was negative for CDC-XM. Two sera were positive for class I Ag in the AMS assay, and the results of CDC-XM were also positive for T-cell. The AMS assay was positive for HLA class II Ag in the five sera. Of these, two sera were positive for B-cell CDC-XM, but CDC-XM was negative in the three sera.

The Luminex-DSA was compatible with the CDC-XM results in 68 sera (85.0%), and there was significant concordance ($\kappa = 0.42$, $p = 0.001$). Comparison of the AMS and Luminex-DSA results with

Table 3

Comparison of transplantation outcomes between positive and negative AMS results in patients with positive Luminex-DSA

	Positive AMS (n = 6)	Negative AMS (n = 6)	p Value
Pretransplantation			
Age (y)	39.5 ± 11.0	45.3 ± 7.7	0.312
Male (%)	4 (66.7)	2 (33.3)	0.567
HLA-mismatch number	3.33 ± 1.37	3.67 ± 1.03	0.644
Re-transplantation (%)	4 (66.7)	1 (16.7)	0.242
Desensitization (%)	5 (83.3)	2 (33.3)	0.242
ABO-incompatibility (%)	1 (16.7)	2 (33.3)	1.00
Posttransplantation			
Acute cellular rejection (%)	1 (16.7)	1 (16.7)	1.00
Acute antibody mediated rejection (%)	3 (50.0)	0	0.182
eGFR at 12 months (ml/min/1.73 m ²)	48.7 ± 2.0	72.6 ± 30.7	0.157
Allograft loss (%)	0	0	—

the CDC-XM results showed that the number of compatible sera was significantly higher for the AMS assay results than for the Luminex-DSA ($p = 0.008$).

3.4. Comparison between the AMS assay Luminex-DSA with the FCXM assay

The AMS assay results were compatible with the FCXM assay results in 72 sera (90%), and there was a significant degree of concordance between the FCXM assay and AMS assay results ($\kappa = 0.50$, $p = 0.001$). Of two sera with AMS positivity against both class I and class II Ags, FCXM assay was positive for both T- and B-cell in one serum, but the other one was negative for FCXM. The AMS assay was positive for HLA class I Ag in two sera and FCXM was also positive for T-cell. Five sera were positive for class II Ag in the AMS assay. Of these, two sera were positive for B-cell FCXM, but the other three sera were negative for FCXM.

The Luminex-DSA was compatible with the FCXM assay results in 68 sera (85.0%). There was a significant degree of concordance between the FCXM and Luminex assay results ($\kappa = 0.46$, $p < 0.001$). Comparison of the number of compatible sera between AMS and Luminex assay results with the FCXM assay results showed that the number of compatible sera was significantly higher for the AMS assay results than for the Luminex-DSA ($p = 0.001$).

3.5. Clinical significance of the AMS assay in patients with positive Luminex-DSA

Table 3 shows clinical characteristics and outcomes in 12 patients with positive Luminex-DSA (Table 3). The sequential data of some patients after desensitization were also summarized in the supplemental Table 1. Of 12 patients with positive Luminex-DSA, six patients also showed the AMS positivity. The patients with positive AMS showed tendency of higher proportion of re-transplantation and desensitization than the patients with negative AMS. Acute antibody-mediated rejection was developed only in patients with AMS positivity ($n = 3$, 50%). The eGFR at 12 months in AMS-positive recipients was lower than in AMS-negative recipients, although it was statistically insignificant.

Table 2

Number of compatible or incompatible sera in the AMS and DSA by single-antigen Luminex assay relative to crossmatch tests

	For CDC-XM			For FCXM		
	AMS	Luminex DSA	p Value	AMS	Luminex DSA	p Value
Compatible	73 (91.3)	68 (85.0)	0.008	72 (90.0)	68 (85.0)	0.001
Incompatible	7 (8.8)	12 (15.0)		8 (10.0)	12 (15.0)	

Note: Twenty-one sera were excluded from a total of 101 sera, because of the influence of the pre-transplantation desensitization protocol.

DSA, donor-specific antibody; CDC-XM, complement-dependent cytotoxicity crossmatch; FCXM, flow cytometry crossmatch; AMS, antibody monitoring system.

4. Discussion

The results of our study clearly demonstrate that the AMS assay has high specificity and high positive predictive value to detect Luminex-DSA in the sera from sensitized recipients and that the positivity in the AMS assay was closely associated with positive Luminex-DSA with strong MFI level. Furthermore, the results of the AMS assay were more concordant with those of the CDC-XM and FCXM assays than were those of the single-Ag Luminex assay. These findings suggest that the AMS assay is useful for predicting actual crossmatch results and strong Luminex-DSA in sensitized patients.

In sensitized renal transplantation, cell-based crossmatch has several limitations. It lacks the specificity for HLA antigen, and use of anti-CD20 monoclonal antibody yielded false-positive results. Furthermore, the requirement for viable donor cell might interfere with post-transplantation immunologic monitoring. Therefore, solid-phase assay is a fundamental tool in renal transplantations of sensitized patients [19]. In our study, the AMS assay had a high positive predictive value for Luminex-DSA in sensitized patients, and the positivity of AMS assay was closely associated with Luminex-DSA-positive sera with strong MFI. These findings suggest that the AMS assay is a useful solid-phase assay that might overcome the limitations of the conventional cell-based method.

Several recent investigations have reported that the weak anti-HLA antibody does not necessarily translate into the clinical relevance [9,10,17–20]. In the present study, AMS assay showed the high negative predictive value to detect Luminex-DSA in nonsensitized recipients and Luminex-DSA-positive sera with weak MFI were usually associated with negativity in the AMS assay. Furthermore, the best prediction of AMS positivity was an MFI of approximately 2000 in the ROC curve. These findings suggest that the AMS assay is helpful to differentiate the weak Luminex-DSA and that it might optimize the excessive sensitivity of the Luminex assay.

In two sera from nonsensitized recipients, the AMS assay results were positive but the Luminex-DSA results were negative. We presume that the AMS assay might have produced false-positive results in these sera because the sera were also negative in the CDC-XM and FCXM assays. Nevertheless, the false-positive results were infrequently found in the results of AMS assay using healthy donor and lymphocytes (Supplemental Table S2). Interestingly, the evidence of recent Epstein–Barr virus (EBV) infection and anti-neutrophil antibody was observed in those patients. These findings suggest that the interference from autoantibody or EBV associated antibody could be related to the false-positive results of the AMS assay.

The high sensitivity and specificity of the single-Ag Luminex assay for detecting DSA facilitates virtual crossmatching in renal transplantation. However, multiple studies have demonstrated that virtual crossmatching is limited when predicting the crossmatch results accurately, because the Luminex assay is not donor specific and the crossmatch is not HLA specific [21,23,24]. In our study, the AMS assay had significantly higher compatibility for the crossmatch than the single-Ag Luminex assay. These findings suggest that the AMS assay can improve the accuracy of virtual crossmatching, and should reduce the likelihood of the loss of opportunity for transplantation.

The Luminex platform assay enables the quantification of anti-HLA antibodies and allows risk stratification for adverse immunologic events after renal transplantation. However, the MFI level of Luminex-DSA alone was not enough to obtain better clinical performance, and it is increased when a third-party immune monitoring method is simultaneously positive [9,22]. In this study, we demonstrated that patients with positivity for both AMS assay and Luminex-DSA were more likely to show reduced eGFR and high incidence of acute antibody-mediated rejection than those with positivity for Luminex-DSA only. These findings suggest that the

AMS assay can serve as a method to support the single-antigen Luminex assay for appraising the immunologic risk.

Several findings in our study indicate the clinical advantages of the AMS assay to support the Luminex assay. First, the AMS assay is beneficial for predicting actual crossmatch after desensitization because its results are not influenced by desensitization. Therefore, the AMS assay can help the Luminex assay to determine whether, and when, desensitization can be discontinued. Second, the AMS assay is useful to investigate the donor specificity in patients with different results between Luminex and crossmatch assay. Because the AMS assay is both donor specific and HLA specific, it supports the functional relevance of the Luminex-DSA.

One limitation of this study is that we could not include the larger sample size to interpret the clinical relevance of incompatible results between the AMS assay and single-Ag Luminex assay. In addition, we measured the donor-directed antibody against only HLA-A, -B, and -DR but not against HLA-C, -DQ, and -DP. A study that includes a larger number of sera and patients with complete HLA typing is needed to determine which test is more predictive of allograft outcome.

In conclusion, the AMS assay is useful as a supportive solid-phase method for predicting actual crossmatch and high level of Luminex-DSA in sensitized patients. The AMS assay has the advantages of both the crossmatch and solid-phase assays, and can provide useful information about the immunologic status of renal transplant recipients.

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Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.humimm.2012.01.017](https://doi.org/10.1016/j.humimm.2012.01.017).

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Supplemental Table 2 The results of AMS assay in twenty healthy individuals

Patient	AMS class I	AMS class II
1	negative	negative
2	negative	negative
3	negative	negative
4	negative	negative
5	negative	negative
6	negative	negative
7	negative	negative
8	negative	negative
9	negative	negative
10	negative	negative
11	negative	negative
12	negative	negative
13	negative	negative
14	negative	negative
15	negative	negative
16	negative	negative
17	negative	negative
18	negative	negative
19	negative	negative
20	negative	negative

AMS = antibody monitoring system; AMS class I = AMS

assay against HLA class I antigen; AMS class II= AMS assay

against HLA class II antigen.

Supplemental Table 2 The results of AMS assay in twenty healthy individuals

Patient	AMS class I	AMS class II
1	negative	negative
2	negative	negative
3	negative	negative
4	negative	negative
5	negative	negative
6	negative	negative
7	negative	negative
8	negative	negative
9	negative	negative
10	negative	negative
11	negative	negative
12	negative	negative
13	negative	negative
14	negative	negative
15	negative	negative
16	negative	negative
17	negative	negative
18	negative	negative
19	negative	negative
20	negative	negative

AMS = antibody monitoring system; AMS class I = AMS assay against HLA class I antigen; AMS class II= AMS assay against HLA class II antigen.