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Status quo of histocompatibility testing and prospects for virtual crossmatching within the Korean kidney allocation system: survey of laboratory directors

John Jeongseok Yang[1], Borae G Park[2], Hyun-Woo Choi[3], Eun Young Song[4], Jaeseok Yang[5], Jae Berm Park[6], Eun-Suk Kang[1]

[1]Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea
[2]KODA LAB, Korea Organ Donation Agency, Seoul, Korea
[3]Department of Laboratory Medicine, Chonnam National University Hospital, Chonnam National University Medical School, Gwangju, Korea
[4]Department of Laboratory Medicine, Seoul National University Hospital, Seoul National University College of Medicine, Seoul, Korea
[5]Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Korea
[6]Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

Background: The Korean kidney allocation system (KAS) currently imposes disparities for highly sensitized candidates because it does not account for human leukocyte antigen (HLA) antibody-defined unacceptable antigens. Virtual crossmatch (VXM) may provide a solution by enhancing histocompatibility assessment and improving the efficiency of the overall allocation process. This study surveyed Korean HLA laboratory directors to evaluate current practices, opinions on VXM, and prerequisites for its implementation.
Methods: An electronic survey addressing HLA typing, HLA antibody testing, and VXM concepts was distributed to 48 histocompatibility laboratory directors in September 2024. Responses were received from 31 institutions (64.6% response rate), representing 72.9% of deceased donor kidney transplants performed in Korea in 2024.
Results: All responding institutions conducted HLA typing and physical crossmatch (PXM). Only 61.3% performed single antigen bead assays in-house, and 77.4% reported donor HLA typing at the two-digit serological level. A strong consensus (88.9%) defined highly sensitized candidates as those with calculated panel-reactive antibody >80%, with 66.7% supporting their prioritization. All participants (100%) recognized "improved process efficiency by excluding highly sensitized patients with presumed PXM positivity from the allocation process" (44.3%) as the greatest benefit of implementing VXM, although mean fluorescence intensity cutoffs and testing intervals varied by institution.
Conclusions: Korean HLA laboratory directors broadly support the concept and need for VXM, acknowledging its potential to improve both efficiency and equity for sensitized patients. However, the lack of standardization in HLA testing application and interpretation remains a significant practical challenge. National initiatives and multidisciplinary collaboration are essential before integrating VXM into the Korean KAS.

Keywords: Kidney transplantation; Surveys and questionnaires; Organ allocation; Virtual crossmatch

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Corresponding author: Eun-Suk Kang
Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-ro, Gangnam-gu, Seoul 06351, Korea
E-mail: eskang@skku.edu

HIGHLIGHTS

- This survey-based study assessed the status quo of histocompatibility testing practices in Korea.
- Consensus was noted among histocompatibility testing laboratory directors regarding the definition of a virtual crossmatch and a highly sensitized candidate.
- The study also presents findings regarding further developments and how to implement virtual crossmatching for deceased donor kidney transplantation in Korea.

INTRODUCTION

Equity is a critical factor in organ allocation for deceased donor kidney transplantation (DDKT) [1]. The current Korean kidney allocation system (KAS) prioritizes human leukocyte antigen (HLA) compatibility and ABO compatibility between donors and recipients. However, concerns have arisen regarding disparities among certain blood groups and highly sensitized candidates. These candidates often face repeated positive crossmatches and prolonged delays in transplantation. This situation undermines the efficiency of the overall allocation process and creates additional burdens related to repeated testing and administrative delays.

The definition of highly sensitized candidates varies internationally but is generally based on calculated panel-reactive antibody (cPRA) thresholds. In the United States, candidates with cPRA≥98% are considered highly sensitized and receive prioritization through the KAS (based on the Organ Procurement and Transplantation Network data as of August 1, 2025). The United Kingdom applies a cPRA threshold of ≥85% in its Highly Sensitised Patient (HSP) scheme (NHS Blood and Transplantation Kidney Offering Scheme, 2020). In Australia, the cutoff is generally cPRA≥95%, although some programs use ≥80% (Australia and New Zealand Dialysis and Transplant Registry Annual Report, 2022). Because these patients experience prolonged wait times due to broad HLA reactivity, many allocation systems incorporate virtual crossmatching (VXM) to increase organ offer efficiency and reduce reliance on physical crossmatching (PXM) in this population.

PXM has long been considered the gold standard for evaluating donor-recipient histocompatibility and has effectively minimized the incidence of hyperacute rejection [2–4]. However, PXM is constrained by the time required and the limited number of candidates that can be tested simultaneously, both of which hinder timely decisions about organ acceptability and contribute to the unnecessary discard of valuable organs in DDKT. With the advent of solid-phase assays that detect HLA antibodies with high sensitivity and allele-level specificity, single antigen bead (SAB) assays have become a central testing method [5,6]. Conceptually derived from SAB assays, VXM has been increasingly integrated into allocation systems [7–9], with the goals of reducing cold ischemia time and lowering the rate of false-positive PXM results [10–13]. The cPRA, derived from properly defined unacceptable antigens, estimates the proportion of incompatible donors within a population and predicts the likelihood of positive PXM results based on antibody strength, measured in mean fluorescence intensity (MFI), and antigen specificity [14]. The main benefit of VXM is its ability to provide a more accurate assessment of histocompatibility, thereby improving the likelihood that highly sensitized candidates will achieve a negative crossmatch [15].

The Korean KAS has not yet incorporated VXM into its DDKT program, and substantial groundwork remains before any policy changes can be considered. In this report, we present the results of a survey conducted among HLA laboratory directors overseeing crossmatch programs as part of a research project of the Korean Society of Transplantation. This survey aimed to determine the current status of histocompatibility assessment workflows from a laboratory perspective and to identify the prospects and prerequisites for VXM implementation within the Korean KAS.

METHODS

This study was reviewed and approved by the Institutional Review Board of Samsung Medical Center (IRB No. SMC2024-08-101). Informed consent was waived because of the survey-based study design.

Survey Development

The survey focused on assessing histocompatibility workflows in the context of DDKT and comprised three main sections: (1) HLA typing, (2) HLA antibody testing using SAB assays, and (3) VXM. Although VXM has not yet been implemented in the Korean KAS, strong interest

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in its potential led us to include items exploring the existing consensus on its definition and purpose, as well as the definition of highly sensitized candidates as prospective targets of VXM. Each section contained both mandatory and optional items, with multiple-choice and free-text options allowing respondents to answer according to their institutional practices. The survey was developed in electronic format using Microsoft Forms (Microsoft). The original Korean-language survey form is provided as supplementary material (Supplementary Material 1).

Survey Participants and Distribution of Questionnaires

Survey participants were histocompatibility laboratory directors or practicing physicians who were specialists in laboratory medicine. As of September 2024, 48 laboratories were participating in the external proficiency testing program for histocompatibility testing organized by the Korean Association of External Quality Assessment Service (KEQAS) (https://keqas.org/). The survey was distributed via email with a link to the web-based questionnaire. It was initially sent on September 13, 2024, followed by two reminder notices. The survey platform was closed and finalized on September 30, 2024.

Survey Processing

The survey was designed to assess histocompatibility testing practices at the institutional level. Only surveys in which all mandatory items were completed were considered valid; incomplete responses submitted by the due date were excluded. Data analysis was performed using GraphPad Prism version 10 (GraphPad Software). Responses are presented as percentages (%) and bar charts displaying categorical distributions. Items using 5-point Likert scales ("strongly agree" to "strongly disagree") were analyzed as discrete categories, while responses on 0–10 scales were visualized using a heatmap.

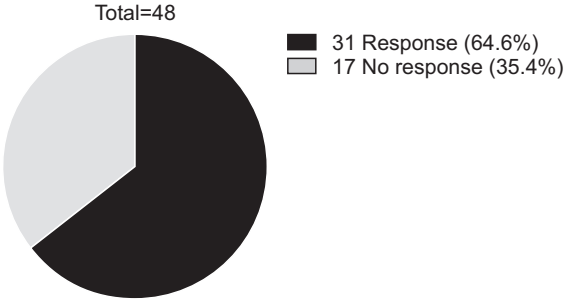

Fig. 1. Participation rate of the survey among histocompatibility laboratories.

RESULTS

Survey Participation

Of the 48 laboratories and institutions contacted, 31 submitted completed survey responses, yielding an overall response rate of 64.6% (Fig. 1). The main reason for non-participation among the remaining 17 laboratories was the lack of involvement in DDKT programs at their institution (47.1%, 8/17), followed by referral status for histocompatibility testing (29.4%, 5/17), and simple non-response (23.5%, 4/17). Notably, the Korea Organ Donation Agency (KODA) operates a histocompatibility laboratory that provides referral services for histocompatibility testing within the DDKT program. The institutions that participated in the survey, including the KODA laboratory, were responsible for HLA testing in 72.2% (588/814) of all DDKT cases performed in 2024, thus providing somewhat greater representativeness than the raw response rate would suggest.

Assessment of Histocompatibility

Participants were asked to identify all test items currently performed at their laboratories. These included HLA typing (at any method or resolution), PXM methods such as complement-dependent cytotoxicity (CDC) and flow cytometry crossmatch (FCXM), and various HLA antibody

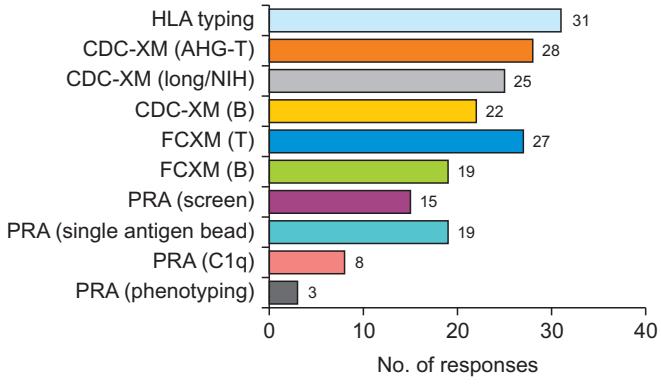

Fig. 2. Histocompatibility test items conducted at participating laboratories. HLA, human leukocyte antigen; CDC-XM, complement-dependent cytotoxicity crossmatch; AHG, antihuman globulin; NIH, National Institutes of Health; FCXM, flow cytometry crossmatch; PRA, panel-reactive antibody.

testing approaches. All participants reported conducting HLA typing and at least one form of PXM at their institutions (Fig. 2). The majority of laboratories reported donor HLA typing at the two-digit resolution (77.4%, 24/31), while a considerable proportion also reported at the four-digit resolution (35.4%, 11/31) (Fig. 3). All laboratories reported testing for HLA-A, -B, and -DR, and most (90.3%, 28/31) also tested and reported HLA-DQB1 loci (Fig. 4). The most commonly performed PXM was antihuman globulin (AHG) phase T cell CDC crossmatch (90.3%, 28/31). However, some institutions did not perform concurrent B cell CDC crossmatch (19.4%, 6/31). A similar discrepancy was observed in FCXM, where eight institutions (25.8%) did not perform B cell FCXM. HLA antibody testing was performed by fewer institutions, with variation in the panel-reactive antibody (PRA) testing methods used. Although SAB assay was the most widely adopted PRA method, only 61.3% (19/31) of institutions performed SAB assays in-house, with the remainder primarily referring samples elsewhere. In terms of reporting intervals, most institutions returned results within 2 weeks. The most frequent testing interval was annual (45.2%, 14/31), although "whenever necessary" was the most common response (58%, 18/31).

All institutions reported that both patient and donor HLA typing information was mandatorily obtained for histocompatibility assessment. Additional sensitization history such as transfusion and pregnancy was also commonly considered. Other relevant information included ABO blood group, prior desensitization treatment, and previous histocompatibility testing results. For donor typing resolution, 77.4% (24/31) of institutions reported results at the two-digit serological equivalent level. Consensus currently favors serological-level reporting for initial typing; however, two institutions indicated that they retrospectively report four-digit or higher results using next-generation sequencing.

Reporting of Human Leukocyte Antigen Antibody Test Results

Participants were asked to indicate all reporting levels used, which resulted in a cumulative number of responses exceeding the total number of institutions (>31). Antibody specificity was reported most commonly at the serological level (28 responses), followed by allele (11 responses), cross-reactive groups (CREG), and epitope or eplet levels (four responses). Four responses indicated that antibody test results were reported in accordance with the resolution of donor HLA typing. The strength of HLA antibodies was reported based on MFI values, although practices varied. Some institutions reported individual MFIs for all antibodies, others reported MFIs in intervals, and some provided MFIs only for donor-specific antibodies (DSAs). Reported MFI values were expressed either as the maximum (peak) MFI or mean (average) MFI, with equal frequency between these two approaches (16 vs. 16). The most common cutoff MFI used for reporting a positive antibody was 1,000, although higher cutoffs (e.g., 3,000) were applied for certain loci such as HLA-C and DRB3/4/5 in some institutions (Fig. 5A). For assigning DSAs, more sensitive thresholds were applied: most institutions used an MFI cutoff of 1,000, while eight used 500 and three reported DSAs even below 500 (Fig. 5B).

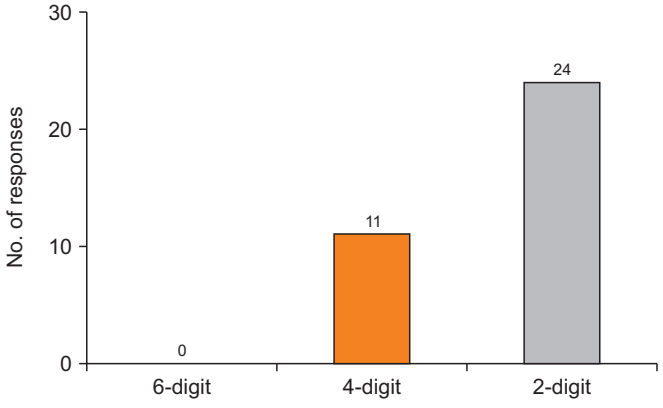

Fig. 3. Reporting resolution of human leukocyte antigen typing for deceased donor kidney transplantation among the participating laboratories.

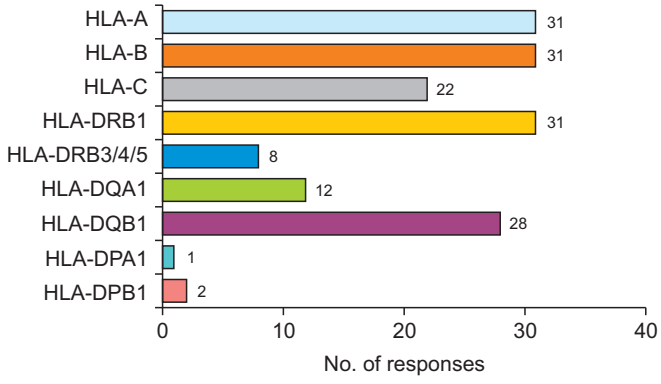

Fig. 4. Reporting loci of human leukocyte antigen (HLA) typing for deceased donor kidney transplantation among the participating laboratories.

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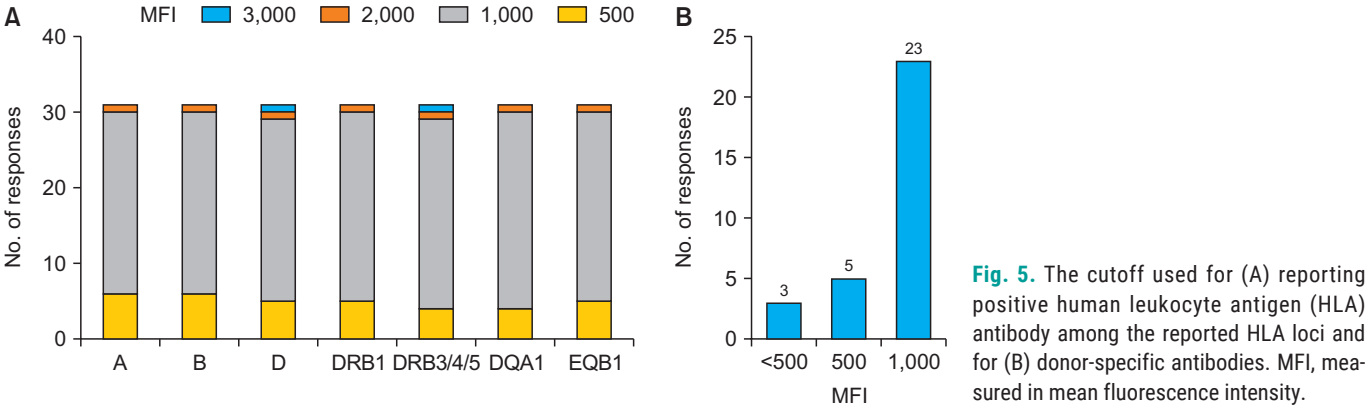

Fig. 5. The cutoff used for (A) reporting positive human leukocyte antigen (HLA) antibody among the reported HLA loci and for (B) donor-specific antibodies. MFI, measured in mean fluorescence intensity.

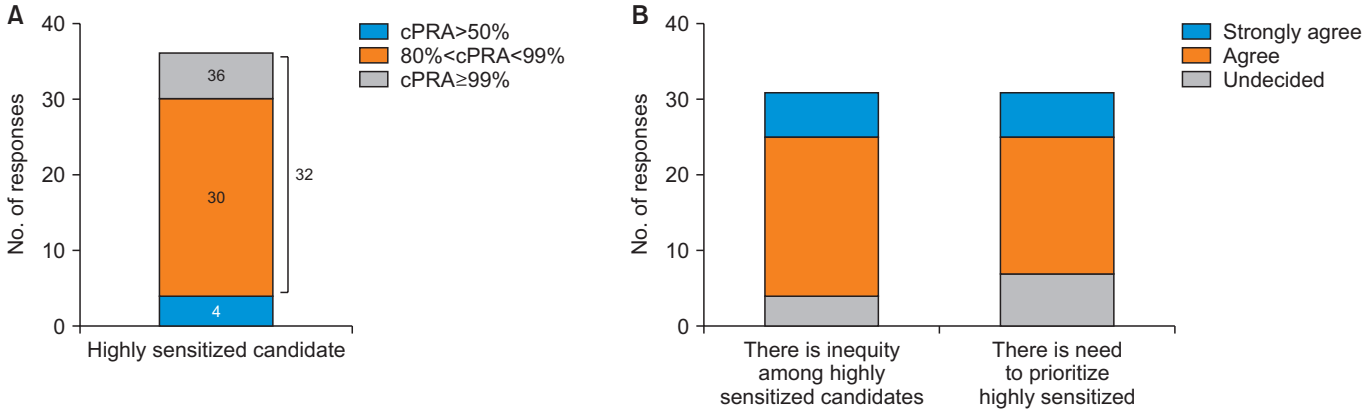

Fig. 6. Opinion on (A) the definition of a highly sensitized candidate, (B) opinion on presence of inequity among highly sensitized candidates and necessity of prioritizing these candidates. cPRA, calculated panel-reactive antibody.

Defining a Highly Sensitized Candidate and Virtual Crossmatching

A large majority of responses (88.9%, 32/36) selected cPRA>80% as the most appropriate definition of a highly sensitized candidate (Fig. 6A). When combining "strongly agree" and "agree," most participants endorsed acknowledging inequity among highly sensitized candidates (87.1%, 27/31) and supported some level of prioritization for these candidates in organ allocation (75.0%, 24/32) (Fig. 6B). All participants (100%) considered VXM to be a simulated donor-recipient histocompatibility assessment used as an auxiliary tool to PXM (Table 1). Nearly half (44.3%) regarded the greatest benefit of VXM as improving workflow efficiency by excluding highly sensitized candidates with a high probability of testing PXM positive.

Cutoffs of Unacceptable Antigens in Virtual Crossmatching and Their Relevance to Physical Crossmatching

An important aspect of VXM is its correlation or concordance with conventional PXM results. When asked about the appropriate level for defining unacceptable antigens using SAB assays, most respondents selected a positive flow cytometry result (48.4%, 15/31), followed by a positive AHG-T CDC crossmatch result (22.6%, 7/31), and a clinically relevant cutoff (29.0%, 9/31) (Fig. 7). Unlike fixed MFI cutoffs or assay outcomes, clinically relevant cutoffs would require extensive testing to confirm the absence of antibodies against specific donor antigens [16].

Potential Limitations of Virtual Crossmatching

The final section of the survey asked participants to identify limitations of VXM and technical challenges associated with SAB assays (Table 2). Based on their selection of the three most relevant limitations, the majority highlight-

Table 1. Consensus on the definition of virtual crossmatching and its anticipated benefits among human leukocyte antigen laboratory directors

Questionnaire	No. of responses
What is the most appropriate definition that describes VXM? (Selection of 1 most relevant option or free written text) (n=31)	
A simulated result of histocompatibility assessment between donor and recipient used in conjunction with PXM methods	31 (100)
An alternative method of histocompatibility assessment to PXM expected to provide equivalent results	0
An improved version of sensitive crossmatch replacing conventional PXM	0
What is the most relevant reason for implementing VXM? (selection of 2 most relevant options) (n=61)	
Improved workflow efficiency by excluding highly sensitized candidates with a high probability of PXM positivity	27 (44.3)
Reduction in crossmatch time, labor and costs	14 (22.9)
Expedited recipient selection leading to improved transplantation outcome	12 (19.7)
Verification of false positive PXM results	6 (9.9)
Reduction in cold-ischemic time and logistics (delays in procurement, transport time, etc.)	2 (3.3)

Values are presented as number (%).
VXM, virtual crossmatching; PXM, physical crossmatching.

Table 2. Human leukocyte antigen (HLA) laboratory directors' survey opinions on the limitations of virtual crossmatching (VXM)

Questionnaire	No. of response (n=86)
What is the most likely limitation of virtual crossmatching? (selection of 3 most relevant options)	
Technical limitation of the SAB assay	21 (24.4)
MFI is incapable of reflecting actual antibody titer and strength	15 (17.4)
Absence of donor HLA-DQB1 genotype information	13 (15.1)
Lack of standardization of SAB reporting, different reporting forms between institutions	13 (15.1)
Absence of allele-level HLA gssenotype information in contrast to allele-level beads used for antibody testing	10 (11.6)
Possible presence of clerical errors during test data input and transfer	8 (9.3)
Insufficient clinical evidence for moving towards VXM	6 (6.9)

Values are presented as number (%).
SAB, single antigen bead; MFI, measured in mean fluorescence intensity .

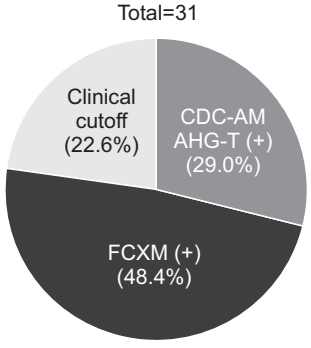

Fig. 7. Consensus on the level of unacceptable antigen defined by single antigen bead, to which a positive virtual crossmatch should correlate. CDC-XM, complement-dependent cytotoxicity crossmatch; AHG, antihuman globulin; FCXM, flow cytometry crossmatch.

ed technical shortcomings of SAB assays (24.4%, 21/86 responses). A major concern was that MFI values may not accurately reflect antibody strength or titer compared with PXM methods such as CDC crossmatch. Additional reported limitations included the lack of donor HLA-DQ typing, the absence of donor and recipient typing at the allele level, and variability in interpreting VXM results. Other issues cited were the absence of standardized SAB reporting, risks of clerical errors during data transfer, and insufficient clinical evidence supporting VXM, all of which must be addressed before VXM can be integrated into practice.

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DISCUSSION

This study provides a comprehensive assessment of the current state of histocompatibility testing practices in Korea, specifically within the context of DDKT, and evaluates readiness for the implementation of VXM. The findings offer important insights into existing workflows, consensus on key definitions, and perceptions regarding the benefits and prerequisites for incorporating VXM into the Korean KAS.

The survey results revealed that all participating institutions adhered to fundamental histocompatibility testing practices, performing both HLA typing and PXM [17–19]. However, significant variability was observed in HLA antibody testing, particularly in the adoption of SAB assays. Although SAB is widely recognized for its high sensitivity and specificity in detecting HLA antibodies, only 61.3% of institutions reported conducting SAB assays on-site. Institutions without on-site testing are limited by turnaround time and by challenges in interpreting results, including assay-related issues such as the prozone effect or interference. This disparity underscores a gap in the standardization of comprehensive HLA antibody testing, which is essential for accurate risk stratification and serves as a critical foundation for conducting VXM [20]. The variation in reporting practices for MFI cutoffs (e.g., 500, 1,000, 3,000) further highlights the need for harmonized protocols in interpreting HLA antibody results.

The study also identified a strong consensus among laboratory directors regarding the definition of highly sensitized candidates, with most selecting cPRA>80%. Agreement on this quantitative threshold is important for developing equitable allocation policies. Furthermore, the recognition of inequities experienced by highly sensitized candidates, along with broad support for prioritization, reflects a clear unmet need for policy adjustments. These findings are consistent with international efforts aimed at improving access to transplantation for highly sensitized candidates, who often face prolonged wait times because of broad donor HLA reactivity leading to persistently positive PXM results. Countries such as the United States, Canada, the United Kingdom, Australia, and Eurotransplant-member nations have incorporated VXM into organ allocation to varying degrees [1,7,8,13]. While some still use PXM, others have transitioned entirely to VXM prior to DDKT. The unanimous acceptance of the provided definition of VXM (100% agreement) demonstrates a clear understanding and acceptance of its core concept among Korean HLA laboratory directors. This shared foundation represents a critical first step toward implementation within the Korean KAS. The most frequently cited benefit of VXM—"improved workflow efficiency by excluding highly sensitized candidates with a high probability of PXM positivity"—directly addresses challenges identified in the introduction, namely the laboratory burden and delays associated with PXM. This indicates that VXM is widely perceived as a means to streamline the allocation process and reduce cold ischemia time, particularly for sensitized patients [11].

The current practice of reporting donor HLA typing at the two-digit serological equivalent level by a majority of institutions (77.4%), despite the availability of high-resolution results, presents a notable challenge for comprehensive VXM implementation. The accuracy of VXM depends on high-resolution donor and recipient HLA typing data to identify DSAs precisely, and in some cases, allele-level resolution is necessary [21,22]. Reliance on predominant serological-level reporting may therefore act as an obstacle to optimal VXM prediction, potentially resulting in missed transplant opportunities or unnecessary PXM procedures. While the inclusion of HLA-DQB1 typing by 90.3% of institutions is a positive finding, further high-resolution typing across expanded loci would significantly enhance VXM accuracy. Achieving this, however, would require expanded insurance reimbursement coverage as well as more widespread use of high-resolution genotyping methods for organ donors. In addition, although the definition of unacceptable antigens is the most important factor, the HLA loci included in cPRA calculations also substantially affect results. A consensus must therefore be established regarding which loci to include—for example, DR51, DR52, and DR53—and whether class I and class II antigens should be calculated separately or collectively remains an additional subject for future discussion.

The survey also highlighted several key prerequisites for VXM implementation, suggesting areas for further development. These include increased utilization and more regular testing intervals for SAB assays, standardization of HLA antibody test reporting, adoption of universal MFI cutoff values, and a potential shift toward routine high-resolution HLA typing for all donors and recipients. Addressing such technical variations and establishing national guidelines will be essential to achieving a successful and equitable VXM program. For example, DDKT programs using VXM in other countries require high-reso-

lution HLA genotyping of both candidates and donors and apply vendor-specific MFI cutoffs to define unacceptable antigens. More centralized histocompatibility testing, as implemented in Canada or Australia, has demonstrated success with these approaches, although whether this model can be adapted to the Korean setting remains to be determined.

As also shown by the survey results on limitations of VXM, participants expressed the greatest concern regarding the technical constraints of the SAB assay [23,24]. MFI values are vulnerable to several interferences and limitations inherent to recombinant protein-based immunoassays, and laboratories often attempt to mitigate these issues through methods such as ethylenediaminetetraacetic acid treatment or serial dilutions. Nevertheless, SAB assays remain limited in their role as surrogate measures of HLA antibody specificity and strength. Furthermore, the varying frequencies of antibody testing across institutions—ranging from "annually" to "whenever necessary"—highlight the need for a more standardized, and potentially more frequent, testing schedule for waitlisted candidates to maintain up-to-date antibody profiles, which are critical for ensuring the accuracy and clinical applicability of VXM.

The consensus on the definitions of VXM and highly sensitized candidates was largely consistent—almost unanimous—among members of the histocompatibility community, indicating strong agreement. Although cPRA can be measured with accuracy, establishing a universal cutoff for unacceptable antigens, such as standardized MFI thresholds derived from SAB assay results, remains a critical issue [8,25,26]. Without such standardization, inclusion of all positive antibodies in cPRA calculations may lead to overestimation of cPRA values and false-positive VXM results. This concern is supported by the observations presented in Fig. 8, where negative crossmatches were not expected in candidates with cPRA>80%, according to survey responses. To reconcile these inconsistencies between cPRA values and actual crossmatch outcomes, properly defined cutoffs for unacceptable antigens must be developed and adopted.

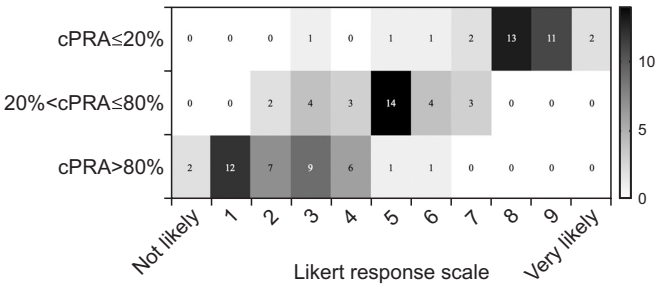

Fig. 8. Opinion on the likelihood of a negative crossmatch result in different ranges of calculated panel-reactive antibodies (cPRA).

The main limitation of this study is its relatively modest response rate (64.6%), which introduces the possibility of bias favoring VXM and reflecting the perspectives of those already interested in revising the Korean KAS. Smaller institutions and laboratories with fewer histocompatibility testing capabilities or limited involvement in DDKT were less inclined to respond, contributing to the lower participation rate. Consequently, the results may overestimate enthusiasm for VXM and perceptions of readiness for its implementation. In addition, the survey focused exclusively on laboratory medicine specialists overseeing histocompatibility laboratories, while excluding valuable input from nephrologists, transplant surgeons, coordinators, and patient communities. Although this study was commissioned by the Korean Society for Transplantation, the perspectives of organ procurement organizations were not directly solicited, and their views on practical challenges may differ from those expressed here. Finally, as with all self-reported surveys, there is inherent potential for differences in the interpretation of questions or inaccuracies in responses. Conducting follow-up surveys to address these limitations, along with organizing public hearings that engage healthcare professionals and relevant experts, should be considered.

Despite these limitations, the study provides a focused perspective on the requirements and directions for integrating VXM into the Korean KAS from the standpoint of histocompatibility testing. Its strength lies in establishing a unified view regarding the definition of highly sensitized candidates. Moving forward, the histocompatibility testing community should prioritize the development of standardized protocols for HLA antibody testing, including consistent use of SAB assays, generally applicable MFI cutoffs for antibody reporting, and feasible testing intervals for transplant candidates. Prospective studies evaluating the clinical and socioeconomic impact of VXM implementation in the Korean DDKT program will also be essential before moving forward. In addition, collaborative efforts among all stakeholders will be critical for considering and guiding the integration of VXM into the Korean KAS.

This survey reveals a strong foundational understand-

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ing and positive disposition towards virtual crossmatch among Korean HLA laboratory directors. There is clear consensus on the need to address inequities for highly sensitized patients and a recognition of VXM's potential to improve workflow efficiency. While technical variations in HLA antibody testing and typing resolution need to be harmonized, the groundwork for VXM integration into the Korean KAS is a work in process. Addressing these prerequisites through national standardization efforts will be essential for successfully realizing the benefits of VXM in optimizing DDKT in Korea.

ARTICLE INFORMATION

Conflict of Interest
No potential conflict of interest relevant to this article was reported.

ORCID
John Jeongseok Yang https://orcid.org/0000-0002-7739-1009
Borae G Park https://orcid.org/0000-0001-9710-9253
Hyun-Woo Choi https://orcid.org/0000-0002-9438-1603
Eun Young Song https://orcid.org/0000-0003-1286-9611
Jaeseok Yang https://orcid.org/0000-0002-5378-7797
Jae Berm Park https://orcid.org/0000-0001-9117-2278
Eun-Suk Kang https://orcid.org/0000-0001-6386-6520

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Author Contributions
Conceptualization: BGP, ESK, EYS, HWC, JJY. Formal analysis: ESK, JJY. Funding acquisition: ESK. Investigation: BGP, ESK, EYS, HWC, JJY. Resources: JBP, JSY. Writing–original draft: ESK, JJY, HWC. Writing–review & editing: all authors. All authors read and approved the final manuscript.

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