## Original Article

# Comparison between complement-dependent cytotoxicity and flow cytometry crossmatches in deceased donor kidney transplantation

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***Abstract:***

***Introduction:*** *Complement-dependent cytotoxicity crossmatch (CDC-XM) and CDC-antihuman globulin-XM (CDC-AHG-XM) have been widely employed to detect donor-specific antibodies in recipients on the waiting list before proceeding with deceased donor kidney transplantation. The flow cytometric crossmatch (FC-XM) has been introduced, and providing increased sensitivity and less time-consumption compared with both assays.* ***Objective:*** *This study aimed to compare the standard CDC-AHG-XM and the three-color FC-XM in deceased donor kidney transplants and to evaluate their sensitivity and specificity.* ***Materials and Methods:*** *Altogether, 365 non-repeated serum samples were included from patients who were candidates for deceased donor kidney transplantation according to the Organ Donation Centre of the Thai Red Cross Society (ODC-TRCS) allocation score. Patient serum samples were crossmatched using CDC-XM, CDC-AHG-XM and FC-XM with T and B lymphocytes obtained from 50 deceased donors. The HLA class I and II antibodies in each patient were also determined.* ***Results:*** *The crossmatch results obtained by FC-XM were compared with the standard CDC-AHG-XM. The sensitivity and specificity of FC-XM in T and B cells were 100 and 100% and 98.86 and 99.41%, respectively. Although FC-XM requires high technical expertise to perform the test, its applications could reduce testing time from 6 to less than 2 hours compared with CDC-AHG-XM. This would be helpful in improving transplant allocation and patient care.* ***Conclusion:*** *In this study, we evaluated the three-color FC-XM and compared the crossmatch results with the routinely used CDC-AHG-XM. The FC-XM is equal to or better than the CDC-AHG-XM because it requires fewer testing steps and is less time-consuming, which is beneficial in deceased donor kidney transplantation.*

***Keywords :*** ● *Complement-dependent cytotoxicity* ● *Flow cytometry* ● *Crosssmatch* ● *Kidney transplantation*

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**นิพนธ์ต้นฉบับ**

# การเปรียบเทียบการทดสอบความเข้ากันได้ด้วยวิธี complement-dependent cytotoxicity กับวิธีโฟลว์ไซโตเมทรีสำหรับการปลูกถ่ายไตจากผู้บริจาคไตสมองตาย

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***บทคัดย่อ***

***บทนำ*** *วิธี complement-dependent cytotoxicity crossmatch (CDC-XM) และ CDC-antihuman globulin-XM (CDC-AHG-XM) ได้นำมาใช้เพื่อตรวจแอนติบอดีในผู้ป่วยรอปลูกถ่ายไตที่จำเพาะต่อผู้บริจาค ก่อนการปลูกถ่ายไตจากผู้บริจาคไตสมองตาย* ***วัตถุประสงค์*** *เพื่อเปรียบเทียบการตรวจ CDC-AHG-XM กับโฟลว์ไซโตเมทรีสามสี (FC-XM) ก่อนการปลูกถ่ายไต รวมทั้งประเมินความไวและความจำเพาะ* ***วัสดุและวิธีการ*** *ซีรัมผู้ป่วยที่ได้คัดเลือกมาตรวจความเข้ากันได้ตามเกณฑ์ของศูนย์รับบริจาคอวัยวะ สภากาชาดไทย จำนวน 365 รายโดยใช้วิธี CDC-XM, CDC-AHG-XM และ FC-XM กับ T และ B lymphocytes ของผู้บริจาคไตจำนวน 50 ราย ทั้งนี้ผู้ป่วยแต่ละรายได้ตรวจแอนติบอดีต่อ HLA class I และ class II แล้ว* ***ผลการศึกษา*** *เมื่อเปรียบเทียบการตรวจความเข้ากันได้ด้วย FC-XM กับ CDC-AHG-XM พบว่า วิธี FC-XM มีความไวและความจำเพาะ เท่ากับ 100 และ 100% และ 98.86 และ 99.41% สำหรับ T และ B cells ตามลำดับ แม้ว่า FC-XM ต้องใช้บุคลากรที่มีประสบการณ์สูงในการตรวจ แต่การประยุกต์ใช้วิธีนี้สามารถลดเวลาในการตรวจจาก 6 ชั่วโมง เป็นน้อยกว่า 2 ชั่วโมง จึงเป็นประโยชน์ต่อการจัดสรรเพื่อการปลูกถ่ายไตและการดูแลรักษาผู้ป่วย* ***สรุป*** *การศึกษานี้ผู้วิจัยได้ประเมิน FC-XM และเปรียบเทียบผลการตรวจความเข้ากันได้กับวิธีตรวจประจำ CDC-AHG-XM การใช้ FC-XM มีผลการตรวจเทียบเท่าหรือดีกว่าการตรวจด้วย CDC-AHG-XM เนื่องจากขั้นตอนการตรวจและเวลาที่ใช้น้อยกว่า ซึ่งมีประโยชน์ในการปลูกถ่ายไตจากผู้บริจาคไตสมองตาย*

***คำสำคัญ :*** ● *โฟลว์ไซโตเมทรี* ● *การตรวจความเข้ากันได้* ● *การปลูกถ่ายไต*

***วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต. 2566;33:179-87.***

## Introduction

The deceased donor kidney transplantation is managed by the Organ Donation Centre (ODC) and the National Blood Centre (NBC) of the Thai Red Cross Society (TRCS). Generally, the ABO blood group, Rh(D) typing, HLA-A, -B and -DR typing, together with the panel reactive antibody (PRA) results of the patients on the waiting list in each transplant center are registered in the ODC-TRCS kidney allocation program.[1] Following the notification from the transplant coordinators of a donor, ABO blood group, Rh(D) typing and HLA typing results provided by the NBC-TRCS are submitted to the kidney allocation program to search for the sequences of patients'scores. The serum samples of patients on the waiting list with the highest overall score calculated from HLA-A, -B and -DR mismatches, PRA and renal waiting time can be chosen for HLA crossmatch with donor T and B lymphocytes. Patients with negative crossmatches might essentially undergo transplantation.[2,3]

Complement-dependent cytotoxicity crossmatch (CDC-XM) has been widely employed for HLA crossmatch, to detect donor-specific antibodies (DSA) in recipients before transplantation. The presence of preformed DSA among patients may cause hyper-acute and acute antibody-mediated graft rejection.[4,5] The addition of antihuman globulin (AHG) to CDC-XM (CDC-AHG-XM) revealed an increase in test sensitivity compared with the standard CDC-XM.[6,7] According to the report of the ODC-TRCS, the duration of testing for those of the above-mentioned assays was approximately five to six hours, starting from receiving the spleen and/or lymph nodes of the deceased donors to the time that the NBC-TRCS laboratory informed the ODC-TRCS of the results. The delayed reporting of results might occur when the laboratory receives multiple donors at the same time.[3] After transplantation, the prolonged cold ischemia time (CIT) may have an impact on the recipients' long-term graft survival and graft failure rates.[7-9] The flow cytometric crossmatch (FC-XM) has been introduced in 1983, and provides increased sensitivity to identify low titers of DSA and non-complement-binding DSA in the recipient's serum bound to the surface of the donor's T and B lymphocytes through using a fluorescently labeled $F(ab')_2$ antihuman IgG[2.11-15] The FC-XM has undergone revisions with three-color analysis,[16] a rapid optimized procedures that constitutes a 96-well tray platform, and additional time-saving protocols such as the Halifax and Halifaster protocols.[17] Related studies revealed that the applications of FC-XM and solid-phase HLA antibody screening techniques, including Luminex single antigen beads (SAB), have increased the sensitivity of antibody detection, leading to improved clinical outcomes.[7,18,19] Currently, the NBC-TRCS laboratory continues performing HLA crossmatches for deceased donor kidney transplantation using the CDC-XM and CDC-AHG-XM. Therefore, this study aimed to compare the standard CDC-AHG-XM and the three color FC-XM in deceased donor kidney transplants and to evaluate their sensitivity and specificity.

## Materials and Methods

### Study population

This study was approved by the Research Ethics Committee, National Blood Centre, Thai Red Cross Society (COA No. NBC 19/2019). Altogether, 365 non-repeated serum samples from patients on the waiting list who were candidates for deceased donor kidney transplantation according to the ODC-TRCS allocation score were included. Patient serum samples were tested with T and B lymphocytes obtained from 50 deceased donors from October 2019 to January 2023. The HLA class I and II antibodies in each patient were detected using a solid-phase immunoassay based on a Luminex platform (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Current and peak sera among patients with HLA antibodies were used for HLA crossmatch by the CDC-AHG-XM and the FC-XM.

**HLA antibody screening for PRA class I and class II antibodies**

The HLA antibody screening was performed on a Luminex platform, according to manufacturer instructions. Briefly, 20 μL of each serum sample were added to each well of a 96-well plate and incubated with 5 μL of LABScreen PRA class I and LABScreen PRA class II beads. Then the test plate was placed on a shaker for 30 minutes at room temperature (RT). Following the incubation, 150 μL of 1X wash buffer were added to each well, covered with a tray seal, and vortexed. The test plate was centrifuged at 1,300 × *g* for 5 minutes. The wash buffer in the wells of the plate was removed by flicking. Thereafter, 200 μL of 1X wash buffer was added to each well of the plate. The plate was covered with a tray seal, mixed, and centrifuged at 1,300 × g for 5 minutes. This step was repeated once. For sample labeling, 100 μL of 1X PE-conjugated anti-human IgG were added to each well, covered with a tray seal, and vortexed. The test plate was incubated in the dark for 30 minutes at RT on a shaker. After incubating, the plate was centrifuged at 1,300 × g for 5 minutes, the tray seal was removed, and the wash buffer was flicked out. The washing step was repeated twice. Then, 80 μL of 1X phosphate buffer solution (PBS) was added to each well, covered with a tray seal and mixed. Finally, samples were read on the LABScan3D™ flow analyzer (One Lambda, Inc., Canoga Park, CA, USA) and analyzed using HLA Fusion Software, Version 3.4 Service Pack (One Lambda, Inc., Canoga Park, CA, USA). The cut-off for reactive samples was based on the mean fluorescence intensity (MFI) value for each HLA antibody. A MFI value equal to or greater than 1,000 was defined as a positive result.

**CDC-XM and CDC-AHG -XM**

The CDC-XM assay was performed using donor lymphocytes and the potential recipient candidate's serum samples. After isolation by Ficoll density gradient centrifugation, donor T and B lymphocytes were incubated with the recipients' serum at 4, 22, and 37°C, respectively. Subsequently, rabbit complement serum was added and activated via the classical pathway of complement activation only by antibodies binding in the first incubation step. The eosin dye was added to stop reactions. The result was positive due to the existence of bound complement-activating cytotoxic antibodies against the donor antigens. The readout of this assay was determined under an inverted phase contrast microscope, and the reactions were defined based on a scoring system with values of 0, 2, 4, 6 and 8, according to the standard protocols. The appearance of dead lymphocytes was visible by a red staining pattern and interpreted as a positive reaction (scores 4, 6 and 8), while vital lymphocytes exhibited a shiny reflective pattern of more than 90% and 80% of cells in a negative reaction (scores 0 and 2).[3,20]

Moreover, the CDC-AHG crossmatch was performed similarly to the CDC-crossmatch method, but by adding of a cell washing step after incubating patient serum with T and B lymphocytes at 4°C, 22°C, and 37°C, and adding antihuman globulin serum (AHG), followed by adding rabbit complement and adding eosin dye.[3,20]

**FC-XM**

The FCXM test was performed in a 96-well U bottom plate; 50 μL of test or positive and negative control serum samples and 25 μL of enriched donor lymphocytes ($2.5 \times 10^5$ cells) were added to each reaction well and incubated at RT for 20 minutes. Cells were washed three times with 200 μL of flow wash buffer at 500 × *g* for 1 minute. Then antibody cocktails consisting of 2 μL of CD3-PC5, 4 μL of CD19-PE, and 0.5 μL of IgG-FITC made up to 50 μL with PBS, were added to cells and incubated for 10 minutes at RT in the dark. After incubating, cells were washed twice more with 200 μL flow wash buffer at 500×*g* for 1 minute, resuspended in 220 μL of cold 1% paraformaldehyde, and readied for acquisition. Flow cytometry was performed on an FC500 flow cytometer (Beckman Coulter, Inc., CA, USA), and analyzed on MXP Software V.2.2 (Beckman Coulter, Inc., CA, USA) on the 1,024 channel scale. The minimum number

of cells for analysis was at least 1,000 events for both T and B cell. The lymphocytes were gated by using dot plots of side scatter (SS) and forward scatter (FS), separated T and B cells using gate of FL4 (CD3-PC5) and FL 2 (CD19-PE), respectively. The FCXM assay results are determined by the median channel fluorescence shift (MCS) of IgG FITC in the sample compared with those of the negative control serum. In this study, the determined cut-off value was calculated by comparing the results between serum samples of known HLA class I and class II antibodies and A pool of 8 non-sensitized patient sera tested against 20 donors' cells to define a cutoff value for positive FCXM as more than 3 SD (> 4.41) and 3.5 SD (> 213.7) shifted to the right for T and B cells, respectively.

**Statistical analysis**

Descriptive analysis was conducted to characterize the baseline demographic data of deceased donors and patients on the waiting list for kidney transplant and expressed in numbers and percentages. The values of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated by using CDC-AHG as a gold standard.

**Results**

The data for this study included 365 patients on the waiting list and 50 deceased kidney donors. Among 365 patients, ages ranged from 9 to 66 years and the most common age group was 51 to 60 years, followed by 41 to 50 years, and 31 to 40 years, consecutively. For deceased donors, ages ranged from 7 to 64 years and the most common age group was 41 to 50 years, followed by 19 to 30 years and 51 to 60 years, successively. Male to female ratio among patients and deceased donors were 201:164 (1.2:1.0) and 40:10 (4.0:1.0), repectively, as shown in Table 1.

The HLA-A, -B and -DR mismatch grades between deceased donors and patients on the waiting list for kidney transplant in this study are shown in Table 2. Among 365 patients, BDR2 was the most common (37.54%), followed by BDR1 (24.38%), BDR3 (21.37%), BDR4 (8.77%), BDR0 (4.93%) and no data available (1.37%), consecutively. In addition, six patients with zero ABDR mismatch (1.64%) were observed.

The distribution of HLA class I and class II antibodies reported as negative (0) or positive in % PRA ranged from 1 to 50, 51 to 80, and 81 to 100, respectively.

**Table 1** Characteristics of the study population

| Characteristic | Patients on the waiting list | | Deceased donors | |
|---|---|---|---|---|
| | N = 365 | % | N = 50 | % |
| **Sex** | | | | |
| - Male | 201 | 55.1 | 40 | 80.0 |
| - Female | 164 | 44.9 | 10 | 20.0 |
| **Age range (years)** | | | | |
| 1-18 | 5 | 1.4 | 8 | 16.0 |
| 19-30 | 25 | 6.8 | 12 | 24.0 |
| 31-40 | 77 | 21.1 | 7 | 14.0 |
| 41-50 | 83 | 22.7 | 13 | 26.0 |
| 51-60 | 124 | 34.0 | 9 | 18.0 |
| >61 | 51 | 14.0 | 1 | 2.0 |
| **Total** | **365** | **100** | **50** | **100.0** |

**Table 2** HLA mismatch of HLA-A, -B and -DR between deceased donors and patients on the waiting list for kidney transplant

| HLA mismatch grade | Patients on the waiting list | % |
|---|---|---|
| Zero mismatch | 6 | 1.64 |
| BDR0 | 18 | 4.93 |
| BDR1 | 89 | 24.38 |
| BDR2 | 137 | 37.54 |
| BDR3 | 78 | 21.37 |
| BDR4 | | |
| BDR4 A0 | 5 | 1.37 |
| BDR4 A1 | 13 | 3.56 |
| BDR4 A2 | 14 | 3.84 |
| No data (others organ transplantation) | 5 | 1.37 |
| Total | 365 | 100.00 |

**Table 3** HLA class I and class II antibodies among 365 patients on the waiting list

| % PRA* | HLA antibodies among patients on the waiting list | | | |
|---|---|---|---|---|
| | Class I | % | Class II | % |
| 0 (Negative) | 268 | 73.42 | 280 | 76.71 |
| 1-50 | 45 | 12.33 | 41 | 11.23 |
| 51-80 | 26 | 7.12 | 17 | 4.66 |
| 81-100 | 17 | 4.66 | 18 | 4.93 |
| No data | 9 | 2.47 | 9 | 2.47 |
| Total | 365 | 100.00 | 365 | 100.00 |

*PRA = panel reactive antibody

The patients' samples with negative results for HLA antibodies were the most common (class I: 268 of 365, 73.72% and class II: 280 of 365, 76.71%). For patients with HLA class I antibodies, 12.33, 7.12 and 4.66% were observed with PRA ranged from 1 to 50, 51 to 80, and 81 to 100, respectively, and 11.23, 4.66 and 4.93% were positive for HLA class II antibodies. No data is available for 2.47% and 2.47% of HLA class I and II antibodies, respectively. (Table 3)

The sensitivity, specificity, positive predictive value, negative predictive value and accuracy of the FC-XM to test patients' serum samples with T cells and B cells from deceased donors were compared with those of the CDC-AHG-XM. We found that the FC-XM exhibited a sensitivity of 100% and 100%, while the specificity of the FC-XM was 98.86 and 99.41% for T and B cells, respectively, as shown in Table 4 and 5.

A discrepancy in crossmatch results was found in six samples of patients on the waiting list, as shown in Table 6. The samples SER055 and SER083 were PRA negative, the CDC-AHG-XM results were negative, while the FC-XM was positive (MCS = 5.20 and 249.01). In the other three samples (SER197, SERn129 and SERn130), CDC-AHG-XM had negative results, while FC-XM had positive results, corresponding to the antibodies specific to donor antigens, including A31, B51 and A11. In addition, CDC-AHG-XM of sample SERn012 was negative, whereas FC-XM had positive results relating to the antibodies specific to donor antigens (DQ7).

**Table 4** Comparison of CDC-AHG-XM and FC-XM among 365 patients tested with T-cells from 50 deceased donors

| FCXM[1] | CDC-AHG crossmatch (T cells) | | |
|---|---|---|---|
| | **Positive** | **Negative** | **Total** |
| Positive | 13 | 4 | 17 |
| Negative | 0 | 348 | 348 |
| **Total** | 13 | 352 | 365 |

[1]Sensitivity: 100%; specificity: 98.86%; positive predictive value: 76.47%; negative predictive value: 100%; accuracy: 98.90%

**Table 5** Comparison of CDC-AHG-XM and FC-XM among 365 patients tested with B-cells from 50 deceased donors

| FCXM[1] | CDC-AHG crossmatch (B cells) | | |
|---|---|---|---|
| | **Positive** | **Negative** | **Total** |
| Positive | 26 | 2 | 28 |
| Negative | 0 | 337 | 337 |
| **Total** | 26 | 339 | 365 |

[1] Sensitivity: 100%; specificity: 99.41%; positive predictive value: 92.85%; negative predictive value: 100%; accuracy: 99.45%

**Table 6** HLA class I and class II antibody specificities found in discrepant crossmatch results between CDC-AHG and FC-XM

| No. | Patient ID | PRA (%) | Antibody specificity | CDC-AHG | FCXM (MCS) |
|---|---|---|---|---|---|
| 1 | SER055 | 0 | Negative | Negative | Positive (5.20) |
| 2 | SER083 | 0 | Negative | Negative | Positive (249.01) |
| 3 | SER197 | 71 | A31[a], B42, B56, B71, B76, B81 | Negative | Positive (5.90) |
| 4 | SERn129 | 35* | A23, A24, A25, A32, B8, B13, B18, B27, B35, B37, B38, B44, B47, B49, B51[b], B52, B53, B57, B58, B59, B63, B64, B65, B67, B71, B75, B77, B78 | Negative | Positive (7.90) |
| 5 | SERn130 | 46 | A11[c], A25, A26, A34, A66, B38, B44, B45, B57, B60, B75, B76, B77 | Negative | Positive (9.80) |
| 6 | SERn012 | 22* | DP1, DQ2, DQ4, DQ6, DQ8, DQ9, DQ7[d], DR7 | Negative | Positive (394.13) |

[a]A31, MFI = 2888; [b]B51, MFI = 5416.58; [c]A11, MFI = 5033.49; [d]DQ7, MFI = 26,366.22

*Antibody specificities in two samples were additionally tested for DSA

## Discussion

The presence of PRA and/or DSA among the recipients against donor HLA-A, -B, and -DR types is a concern due to the risk of hyper-acute kidney allograft rejection in deceased donor kidney transplantation. The CDC-XM is the standard technique for pretransplant evaluation for living-related and deceased donor kidney transplants.[4,20] A recent study by Chinbodi et al. revealed that the CDC-XM and CDC-AHG-XM were used to determine the HLA compatibilities between deceased donor T and B lymphocytes and serum samples of recipient candidates on the waiting list. The limitations of both tests were due to their testing requirements for a high number of lymphocytes with good cell viability, being time-consuming, and revealing more variations in test performance among personnel.[3]

In this study, we compared the HLA crossmatch results between the CDC-AHG-XM and FC-XM among 365 patients on the waiting list and 50 deceased kidney donors. The ratio of male and female among patients and deceased donors was 1.2:1.0 and 4.0:1.0, respectively, and their age ranges in both groups were similar to related studies reported by Ounjai, et al. in 2019 and Srisuddi A, et al. in 2022.[21,22] The FC-XM protocol used in this study was similar to that used in a related study,[16] where a 96-well plate was used instead of test tubes to reduce the cost of reagent and testing time. Moreover, in our protocol, anti-CD3-PerCP, as reported in a recent study using Halifax FC-XM,[17] was replaced by anti-CD3-PC5 without any interference in testing results. From this comparative study, it appears that FC-XM provided an overall sensitivity of 100% for both T and B cell crossmatches, compared to CDC-XM and CDC-AHG-XM confirming other findings.[11,12,16]

Regarding the discrepant results between CDC-AHG-XM and FC-XM among six patients, (two samples with negative PRA and CDC-XM), one patient sample (SER055) might be due to a false positive reaction by FC-XM, while in the other case (sample SER083), DSA did not pre-exist but developed after kidney transplantation (unpublished data). Another four cases with negative CDC-AHG-XM, DSA were demonstrated in the PRA class I and class II testing, agreeing with the positive FC-XM results.

Finally, this study confirms that FC-XM is an alternative to CDC-AHG-XM. Even though the flow cytometers are complex instruments, expensive and require high technical expertise, the FC-XM could reduce testing time from six hours to less than 2 hours with fewer testing steps and technical variations compared with the CDC-XM and CD-AHG-XM. This would be helpful in reducing turnaround time without compromising assay performance and improving transplant allocation and patient care.

## Conclusion

In this study, we evaluated the three-colored FC-XM and compared the crossmatch results with the routinely used CDC-AHG-XM. The FC-XM is equal to or better than the CDC-AHG-XM because it requires fewer testing steps and is less time-consuming, which is beneficial in deceased donor kidney transplantation.

## References

1. *O-Charoen R, Kupatawintu P. Selection criteria for transplantation. In: Jirasiridham S, editor. Textbook of kidney donation for transplantation. Bangkok: Krungthepvejchasarn; 2001. p. 14-21.*
2. *O-Charoen R, Kupatawintu P, Sinsiri S, Salee S, Tatawatorn A, Nathalang O, et al. Preliminary results of selection criteria for cadaveric kidney transplantation by the Thai Red Cross. Transplant Proc. 2000;32:1574-5.*
3. *Chinbordee K, Kukhuntod D, Tatawatorn A, Phiencharoen S, Attajarusit Y, Dhitavat V, et al. Pre-transplant factors influencing HLA crossmatch in deceased donor kidney transplantation. J Hematol Transfus Med. 2020;30:247-53.*
4. *Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. N Engl J Med. 1969;280:735-9.*
5. *Kissmeyer-Nielsen F, Olsen S, Petersen VP, Fjeldborg O. Hyper-acute rejection of kidney allograft, associated with pre-existing humoral antibodies against donor cells. Lancet. 1966;2:662.*
6. *Tait BD, Susal C, Gebel HM, Nickerson PW, Zachary AA, Claas FH, et al. Consensus guidelines on the testing and clinical management issues associated with HLA and non-HLA antibodies in transplantation. Transplantation. 2013;95:19-47.*

7. *Mahoworld GK. The CDC crossmatch in the era of flow cytometric crossmatch and single antigen beads. Braz J Nephrol. 2021;43:299-300.*
8. *Irish WD, Ilsley JN, Schnitzler MA, Feng S, Brennan DC. A risk prediction model for delayed graft function in the current era of deceased donor renal transplantation. Am J Transplant. 2010;10:2279-86.*
9. *Ponticelli CE. The impact of cold ischemia time on renal transplant outcome. Kidney Int. 2015;87:272-5.*
10. *Kayler L, Yu X, Cortes C, Lubetzky M, Friedmann P. Impact of cold ischemia time in kidney transplants from donation after circulatory death donors. Transplant Direct. 2017 23;3:e177. doi: 10.1097/TXD.0000000000000680.*
11. *Garavoy MR, Rheinschmidt MA, Bigos M, Perkins H, Colombe B, Feduska N, et al. Flow cytometry analysis: a high technology crossmatch technique facilitating transplantation. Transplant Proc. 1983;15:1939-44.*
12. *Bray RA, Lebeck LK, Gebel HM. The flow cytometric crossmatch. Dual-color analysis of T cell and B cell reactivities. Transplantation. 1989;48:834-40.*
13. *O'Rourke RW, Osorio RW, Freise CE, Lou CD, Garovoy MR, Bacchetti P, et al. Flow cytometry crossmatching as a predictor of acute rejection in sensitized recipients of cadaveric renal transplants. Clin Transplant. 2000;14:167-73.*
14. *Karpinski M, Rush D, Jeffery J, Exner M, Regele H, Dancea S, et al. Flow cytometric crossmatching in primary renal transplant recipients with a negative anti-human globulin enhanced cytotoxicity crossmatch. J Am Soc Nephrol. 2001;12:2807-14.*
15. *Koktathong K, Vejbaesya S, Bejrachandra S, Pattanapanyasat K. Flow cytometric crossmatch for kidney transplantation. J Med Assoc Thai. 2005;88:769-74.*
16. *Chamsai S, Kittisares K, Thanaketpaisarn Y, Ngamthawornwong S, Koktathong K. Evaluation of the three-colored flow cytometric crossmatch assay in living-related kidney transplantation. J Hematol Trasfus Med. 2018;28:239-48.*
17. *Liwski RS, Greenshields AL, Conrad DM, Murphey C, Bray RA, Neumann J, et al. Rapid optimized flow cytometric crossmatch (FCXM) assays: the Halifax and Halifaster protocols. Hum Immunol. 2018;79:28-38.*
18. *Ilham MA, Winkler S, Coates E, Rizzello A, Rees TJ, Asderakis A. Clinical significance of a positive flow crossmatch on the outcomes of cadaveric renal transplants. Transplant Proc. 2008;40:1839-43.*
19. *Orandi BJ, Garonzik-Wang JM, Massie AB, Zachary AA, Montgomery JR, Van Arendonk KJ, et al. Quantifying the risk of incompatible kidney transplantation: a multicenter study. Am J Transplant. 2014;14:1573-80.*
20. *LeFor WM.ASHI laboratory manual. 4th ed. New Jersey: American Society of Histocompatibility and Immunogenetics; 2000.*
21. *Ounjai S, Ponraweethitikorn P, Kanunthong S, Srisuddee A, Phiencharoen S, Kupatawintu P, et al. HLA-A, -B, and -DR frequencies in deceased kidney donors of the Organ Donation Centre, Thai Red Cross Society. J Hematol Transfus Med. 2019;29:175-81.*
22. *Srisuddee A, Tupmongkol T, Thongpaknam S, Phiancharoen S, Kupatawintu P, Nathalang O, et al. HLA mismatch between deceased donors and kidney transplant recipients: a retrospective study from January 2019 to December 2021. J Hematol Transfus Med. 2022;32:193-200.*