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**Review**

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# Why Crossmatch Tests are Very Important and What Do They Tell Us?

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## Abstract

Organ transplantation is a very complex procedure that can save lives. It is a very useful procedure if the concepts of immunology, gained through bitter experience over the years, are kept in mind during practice. Allogeneic sensitization due to previous exposure(s) to alloantigens can hamper the procedure and remains the main obstacle to organ transplantation. Allosensitization and its level in a patient can be revealed before transplantation using the crossmatch test performed by incubating the patient's serum with the possible donor's lymphocytes in a laboratory environment. Crossmatch tests are routinely used prior to transplantation to prevent acute humoral reactions to the allograft tissue following reperfusion and also for long-term monitoring of the patient's humoral status during the pre- and post-transplantation periods.

**Keywords:** Transplantation, crossmatch test, sensitization

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## INTRODUCTION

Crossmatch tests are used in many areas of medicine. In the context of transplantation, crossmatch tests have widely been used for evaluating the immunological and anamnestic responses of the recipient to the graft antigens and vice versa as well as the response of the allograft to the recipient. Cell-to-cell crossmatch and serum-to-cell crossmatch have commonly been used to determine graft and patient compatibility. Crossmatch tests can be used for monitoring the patient's humoral status during and after transplantation. Practically, patients' possible humoral reactions against possible donor antigens are in focus. Positive crossmatch test results, even at a low positivity, indicate that the antigens have at least once been introduced in the body, that they have been processed and stored in the immune memory, and that they have the potential for inducing future humoral reactions and injuries. During the pre-transplantation period, crossmatch tests can be performed against a specific donor to detect possible sensitizations and presence of antibodies (Abs) specific to the donor and also the overall general population to generate the % panel-reactive antibodies (% PRA) that represents the allosensitization level of the recipient. Organ failure patients who require support for failing organ function are usually treated via very complex medical procedures, including artificial organ replacement systems, transplantation, and/or multiple transfusions to extend their survival; therefore, they usually become sensitized to many alloantigens in the population they live within. Sensitizing antigens when encountered and accepted as important by the host immune system are recorded as an immune memory in B cells, similar to what occurs in vaccination. Currently, sensitization is an ever increasing problem for the patients waiting for transplantation. In recent years, owing to the shortage of good matched donor organs, there has been an increasing interest in overcoming the problem of allosensitization through desensitization procedures that turn sensitized patients to transplantable cases. The level of desensitization is measured and monitored via crossmatch tests following particular desensitization procedures to save the graft from humoral injury (1-3).

Acquired immune reactions post transplantation are antigen-specific reactions to particular allograft antigens, which can lead to acute rejection. The most effective and active arm of acquired reactions is humoral, not cellular, through anti-human leukocyte antigen (HLA) Abs against particular graft antigens. To perform safe organ transplantation, collection of data that will explain the past and current immune status of the patient to accurately predict the fate of organ being transplanted is a prerequisite. The reaction against allogenic tissue is mostly immunogenic, i.e., overall inflammatory, in nature. Innate and acquired immune reactions begin to cooperate immediately post reperfusion and work synergistically to remove the foreign tissue rapidly, even though its function is life saving for the body. If immunosuppressive therapy is not orchestrated properly and remains ineffective and if immune cell functions are not suppressed to comply with the rules of tolerance, the end result is the loss of a great opportunity of survival (4, 5). Tolerance is survival of both the allograft and patient, without reacting against each other. This state is usually achieved with proper graft and patient histocompatibility matching as well as via pharmacological immunosuppression, which realizes silencing of the immune cells and their specific reactions against the graft alloantigens (5).



The immune system, evolved and organized with very painful experiences over hundreds of years, records important information in memory B cells and stores it as memory for the second set reaction. Upon encountering the same antigens again, memory B cells react directly, without T cell assistance, and begin to produce specific Abs spontaneously. When the antigen–antibody complexes are formed, the complement system is activated through the classical way, and the allograft is destroyed; this is the second set reaction (1, 2). Second set reaction is mediated mainly through antigen-specific immunoglobulin (Ig) G-type Abs, and they have a long half-life in the circulation. There are four main IgG Ab subgroups. IgG1 and IgG3 subgroup Abs set and accelerate the immune reactions up and can effectively activate the complement cascade. IgG2 and IgG4 subgroup Abs are rather tolerogenic and do not activate the complement system as effectively as do IgG1 and IgG3. These class differentiations are affected and routed mostly via influences of the local environmental cytokine-mediated factors present around the allograft region, depending on local effects of cytokines, including an inflammatory rejection reaction, a certain tolerogenic effect, and even deceleration of an immune reaction (6, 7). We have to keep in mind that Abs against specific foreign antigens are produced by the acquired immune system, and even though if they do not lead to significant complement activation for effective cytotoxicity, they can still induce opsonization as a low-intensity reaction or subclinical chronic immune reactions that can harm the graft in the long run (4, 5).

Ischemia/reperfusion injury following reperfusion, which is unavoidable during vascularized organ transplantation, leads to severe inflammation due to activation of components of the innate immune system, thus injuring the vascular endothelium of the graft. The inflamed endothelium becomes highly antigenic since endothelial cells are immune cells in nature. These cells are rich in major histocompatibility complex (MHC) antigens under inflammation and present those antigens to the host's acquired immune system (5, 8-11). When the patient is already sensitized, the circulating recipient blood is rich in allo-Abs against the alloantigens, which the graft already expresses; this leads to antibody–antigen binding and complement activation, which in turn activates the classical cascade leading to a series of harmful reactions. These reactions inflict serious additional injuries to the endothelial tissue of the graft as well as the functional parenchymal cells. Depending on the intensity and severity of this endothelial injury, immune activation triggers another innate system, and the coagulation cascades are activated; circulation in the graft stops, and the graft becomes gangrenous. If the immune reaction is less severe at the beginning as well as latter, the acquired immune reaction can be adequately suppressed via pharmacological means; this way, the reaction does not accelerate and remains slow and the slightly injured tissue may be repaired by live graft cells, leading to healing of the graft and its functioning as expected (5). In case of previous sensitization, the recipient will react to the recorded alloantigens immediately after reperfusion as a second set reaction, which is an accelerated response and cannot be halted. Modern powerful prophylactic immunosuppression techniques can prevent delayed type IV first set immune reactions; however, these are not effective against the type of immune cells with memory functions. Abs against the graft antigens, sooner or later, will destroy the allo-organ and return the patient to end-stage organ failure (11, 12).

Abs initiate all alloreactions against the allograft tissue in the recipient body according to the humoral theory. Sensitized patients show lower graft survival rates, as indicated in almost all multicenter studies, even if they are transplanted upon a negative crossmatch test result on a given date (13). Currently, crossmatch tests are widely used to evaluate humoral immunity rather than cellular immunity, and considering the humoral theory of rejection, cell-to-cell immunity tests are very rarely used (14-20). From this point of view, since sensitization and its level are the ultimate predictors during transplantation, crossmatch tests are performed at the beginning to offer an opportunity for correct matching of the allograft and patient as well as to provide information for the potential long-term fate of the allograft via humoral immune monitoring. Crossmatching is a highly strategic test with a particular meaning, and it can be repeated several times according to the indications and implications for future graft survival.

Pre-transplantation understanding of the true sensitization state is essential for achieving a good outcome in transplantation practice. Since importance of sensitizations has been historically demonstrated through bitter experiences, many effective and comprehensive crossmatch test have evolved and many re-

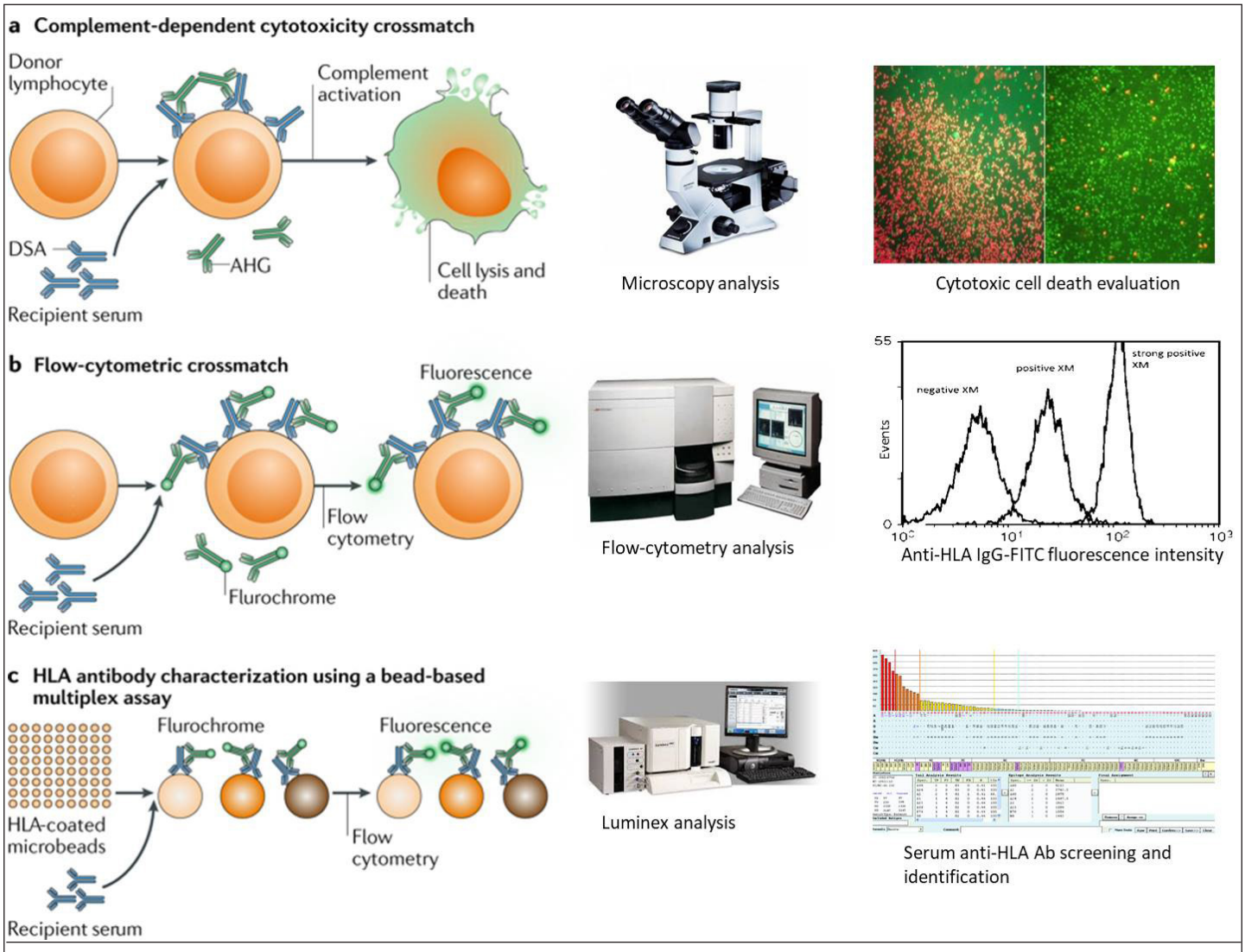


**Figure 1. a-c.** Schematic illustration on the basis of experimental stages, data collection, and results on the (a) CDCXM, (b) FCXM, and (c) Luminex assays. (Modified from Montgomery RA et al., Nature Reviews Nephrology 14, 558–570 (2018)).

searchers are still working on perfecting and increasing the reliability of these test (4, 6, 7, 12, 13). Theoretically, crossmatch tests have primarily been performed by two methods complementing each other using different antigen sources (Figure 1).

Patient serum in which the allo-Abs are searched for is crossmatched using either of the following assays:

1. Live donor lymphocytes as target antigens, called "cellular" or "cell-based" assays
2. A solid surface or ready-to-use microbeads, fitted with donor lymphocyte lysates or known MHC antigens, called "solid-phase" assays

Cell-based assays serologically demonstrate the complement-dependent donor lymphocyte lysis under the microscope (CDCXM) or using fluorescent-tagged anti-human IgG Abs, followed by the detection of allogeneic Abs in the patient serum (FCXM) using flow cytometry.

Solid-phase crossmatch test detect donor-specific allo-Abs in patient serum. It can be performed as a panel study-% PRA-using a set of possible HLA antigens in the population to demonstrate anti-HLA Abs present in the serum of a patient in the waiting list. Solid-phase assays are preferably performed for revealing the allogeneic sensitization level. During screening, a large number of patients' serum samples are tested and anti-HLA Abs are characterized (5, 16). Knowledge of patient characteristics, their sensitization status, and particular antigens they are sensitized to is very important before transplantation for proper matching while they are still in the national waiting list. This may increase their chance to find a donor who presents no HLA antigens the patient is sensitized to. Theoretically, with the aid of this information, the graft can be sent to the most matching remote patient in the waiting list without the need of performing the final actual pre-transplantation crossmatch test. The procedure, called "virtual crossmatch," saves time and allows for share and sending the allograft to remote areas and transplant it with a short cold ischemia time (3, 15, 18).

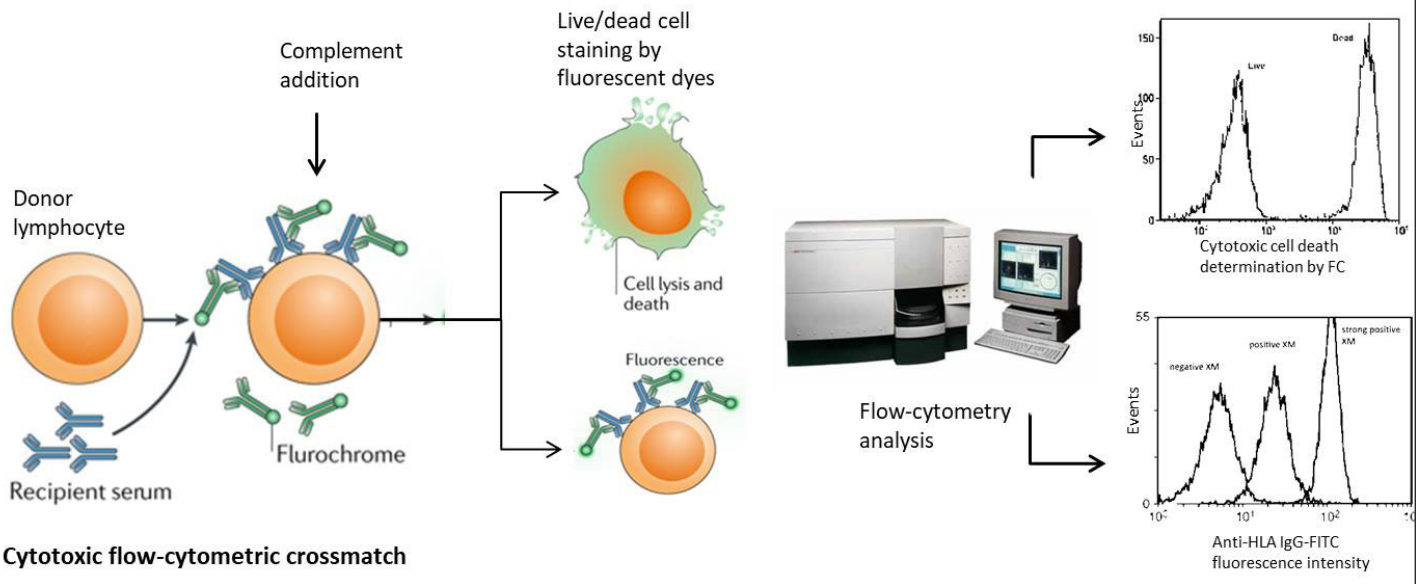


**Figure 2.** Schematic illustration on the basis of experimental stages, data collection, and results of the cFCXM assay. (Modified from Montgomery RA et al., Nature Reviews Nephrology 14, 558–570 (2018)).

In today's transplantation practice, most cell-based crossmatch tests are performed as CDCXM and provide visual evidence of sensitization and percent cytotoxicity. Serological crossmatch test, in which the complement is used, helps measure cytotoxicity visually. However, the decision is a subjective opinion since the result is read by a specialist in charge on that specific day, and the percentage of stained dead cells is calculated by counting them according to their morphology and color in the microscopic area being observed by the specialist. Another popular cell-based crossmatch test is performed using flow cytometry and fluorescent-tagged anti-human Abs. Flow cytometry is a computerized version of fluorescent microscopy in principle. It detects the presence and calculates the intensity of allo-Abs in the recipient's serum. However, since the complement is not used, it does not reveal cytotoxicity. Because the number of fluorescent-tagged lymphocytes is counted electronically, sensitivity of this assay is several times higher than that of the visual serological CDC method. Therefore, flow cytometric crossmatch is statistically more reliable. These two experiments take over 4 h in a laboratory to obtain reliable and detailed results. Flow cytometric crossmatch electronically measures the number of IgG-type allo-Abs attached to the surface of the flowed donor lymphocytes when the sample is passed under a laser beam using a computer program; therefore, it is highly objective. The cells with fluorescent Abs counted by the device are in thousands; thus, the results of this method are much more significant than those of the serological method. Since no complement is used in the flow crossmatch assay, although the Abs attached to the surface of the donor lymphocytes are detected, percent cytotoxicity and presence of complement-binding Abs cannot be determined. In routine practice, many tissue typing laboratories perform both serological and flow cytometric crossmatch tests together to obtain the most necessary information (7). A specialist clinician is aware of the existence of allo-Abs but cannot determine the cytotoxicity. Another flow cytometric test combining both methods (CDC+FCXM) was recently developed—cytotoxic flow cytometric crossmatch (cFCXM); this assay measures the level of allo-Abs and, with complement usage, determines their cytotoxicity in the same experiment (Figure 2). It is a functional test, resembling a serological cytotoxicity test performed using a flow cytometer. The advantage of this method is that it provides more information than both serological and flow cytometric crossmatch tests alone within a single experiment, saves time, and helps reduce the cold ischemia time. Moreover, since it is performed using flow cytometry, thousands of cells are counted and the data obtained are more reliable and significant (8).

These pre-transplant serological and flow cytometric cell-based crossmatch tests are designed to prevent hyperacute humoral injuries and/or possible second set anamnestic reactions against the graft. Availability of information on sensitization memory in advance may increase allograft survival through proper matching and immunosuppression. Studies on the humoral theory have revealed that second set reactions are not rare. Transplant patients with a history of alloimmunization and negative pre-transplant crossmatch test results may harbor sleeping memory B cells, which may become activated rapidly and become plasma cells upon antigen re-encounter after reperfusion (second set reaction); this cannot be prevented by crossmatch tests alone. In this case, the potential of the recipient's anamnestic reactions generated through their immune memory may lead to severe second set humoral rejection reactions following transplantation. It is almost the same reaction principle expected in vaccination reactions. Currently available methods to detect the presence and activity of recipient

HLA-specific memory B cells are scarce and insufficient in quantifying the complete donor-specific memory B cell response following transplantation. There have been an increasing number of articles on this topic over the recent years. Studies have shown that alloreactive memory B cell profiling provides more information on the recipient's allosensitization in addition to the information detected by the serum Ab screenings and crossmatch assays (21). Therefore, we need to gain information on the potential of memory B cells for better long-term graft survival. It is a very useful idea to uncover the possible immune memory status and potential of the recipient to prevent second set reactions (22).

## CONCLUSION

Therefore, to gain a better understanding of the patient's immune memory status for extending graft survival, research to establish better pre-transplant crossmatch tests is an open-ended and ever-improving subject.

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## REFERENCES

1. Vaughan R, Shaw O. How much donor human leukocyte antigen-specific antibody is too much for a renal transplant? Transplantation 2008; 85: 1081-2. [CrossRef]
2. Schinstock CA, Gandhi MJ, Stegall MD. Interpreting anti-hla antibody testing data: a practical guide for physicians. Transplantation 2016; 100: 1619-28. [CrossRef]
3. Schlaf G, Apel S, Wahle A, Altermann WW. Solid phase-based cross-matching as solution for kidney allograft recipients pretreated with therapeutic antibodies. Biomed Res Int 2015; 2015: 587158. [CrossRef]
4. Gautreaux M, D. Histocompatibility Testing in the Transplant Setting in Kidney Transplantation, Bioengineering and Regeneration. 1st ed. Academic Press 2017. [CrossRef]
5. Ata P. Bağışıklık Sistemi ve Antikorlar. Titiz MI, editor. Laboratuvardan Kliniğe Transplantasyon Pratiği. 1st ed. Tekirdağ 2017.
6. Hönger G, Hopfer H, Arnold ML, Spriewald BM, Schaub S, Amico P. Pretransplant IgG subclasses of donor-specific human leukocyte antigen antibodies and development of antibody-mediated rejection. Transplantation 2011; 92: 41-7. [CrossRef]
7. Ata P, Canbakan M, Kara M, Özel L, Ünal E, Titiz M. Serum flow cytometric C1q binding antibody analysis of renal recipients with low levels of sensitization. Transplant Proc 2012; 44: 1652-5. [CrossRef]
8. Bilgen T, Ata P, Tozkir J, Tozkir H, Titiz MI. Cytotoxic Antibody detection by means of flow-cytometric cross-match. Transplant Proc 2017; 49: 440-4. [CrossRef]
9. Bonventre JV, Weinberg JM. Recent advances in the pathophysiology of ischemic acute renal failure. J Am Soc Nephrol 2003; 14: 2199-210. [CrossRef]
10. Requião-Moura LR, Durão MeS, Tonato EJ, Matos AC, Ozaki KS, Câmara NO, et al. Effects of ischemia and reperfusion injury on long-term graft function. Transplant Proc 2011; 43: 70-3. [CrossRef]
11. Kosieradzki M, Rowiński W. Ischemia/reperfusion injury in kidney transplantation: mechanisms and prevention. Transplant Proc 2008; 40: 3279-88. [CrossRef]
12. Terasaki PI, Cai J. Human leukocyte antigen antibodies and chronic rejection: from association to causation. Transplantation 2008; 86: 377-83. [CrossRef]
13. Terasaki PI. A personal perspective: 100-year history of the humoral theory of transplantation. Transplantation 2012; 93: 751-6. [CrossRef]
14. Platt JL, Cascalho M. B Cells in transplantation of rat, mouse, and man. Transplantation 2018; 102: 357-8. [CrossRef]
15. Piazza A, Ozzella G, Poggi E, Caputo D, Manfreda A, Adorno D. Virtual crossmatch in kidney transplantation. Transplant Proc 2014; 46: 2195-8. [CrossRef]
16. Koenig A, Mariat C, Mousson C, Wood KJ, Rifle G, Thaunat O. B Cells and Antibodies in Transplantation. Transplantation 2016; 100: 1460-4. [CrossRef]
17. Morath C, Opelz G, Zeier M, Süsal C. Prevention of antibody-mediated kidney transplant rejection. Transpl Int 2012; 25: 633-45. [CrossRef]
18. Roelen DL, Doxiadis II, Claas FH. Detection and clinical relevance of donor specific HLA antibodies: a matter of debate. Transpl Int 2012; 25: 604-10. [CrossRef]
19. Nascimento E, Fabreti de Oliveira RA, Maciel MD, Pereira AB, das Mercês de Lucas F, Salomão-Filho A, et al. Kidney transplantation: evaluation and clinical outcome of 237 recipients at low, medium, high, or strong immunological risk of rejection. Transplant Proc 2014; 46: 101-7. [CrossRef]
20. Mizutani K, Terasaki P, Hamdani E, Esquenazi V, Rosen A, Miller J, et al. The importance of anti-HLA-specific antibody strength in monitoring kidney transplant patients. Am J Transplant 2007; 7: 1027-31. [CrossRef]
21. Karahan GE, Eikmans M, Anholts JD, Claas FH, Heidt S. Polyclonal B cell activation for accurate analysis of pre-existing antigen-specific memory B cells. Clin Exp Immunol 2014; 177: 333-4. [CrossRef]
22. Karahan GE, Krop J, Wehmeier C, de Vaal Yvonne JH, Langerak-Langerak J, Roelen DL, et al. An Easy and Sensitive Method to Profile the Antibody Specificities of HLA-specific Memory B Cells. Transplantation 2019; 103: 716-23. [CrossRef]