References

A Tool for Enhanced Risk Assessment
Identify patients at risk for acute rejections

The last decades there has been a substantial increase in short-term and long-term survival of transplanted grafts from both living and diseased donors (1). Nevertheless, acute rejection affecting long-term graft survival remains a significant problem. Despite modern immunosuppressive therapies and extensive routine testing for HLA-reactive antibodies, acute rejections still occur in almost 15% of transplant patients (2).

A multicenter study of XM-ONE®, an endothelial crossmatch test, demonstrated that XM-ONE® detects patients at risk of acute rejections, even in cases where conventional lymphocyte crossmatch tests are unsuccessful in detecting donor-specific antibodies (3). XM-ONE® positive patients had earlier and more severe rejections resulting in a sustained increase in serum creatinine.

The long-term consequences of an acute rejection are being debated. Some say it shortens the graft half-life.
Good decisions are based on good information

XM-ONE® improves the basic data for decision-making

Enhancing the crossmatch – from bench to bedside value

The discovery of histocompatibility antigens and identification of HLA specificity has played a central role in first matching recipients with donors and thereafter in assessing the risk of the actual transplantation. However, HLA is not the only antigen thought to affect the long-term survival of the transplanted graft. It is well recognized that antibodies to non-HLA also have a major impact on transplantation success.

XM-ONE® is an endothelial cell based crossmatch test that, apart from providing data on HLA antibodies, brings valuable information on non-HLA antibodies. XM-ONE® is the only commercially available cell based crossmatch test. The test, which is FDA registered in the US and CE marked in EU, provides the laboratory with a tool that ensures reproducible crossmatch data in donor-recipient decision making.
Since the introduction of modern immunosuppressive therapies in the beginning of the 80’s organ transplantation has evolved tremendously enabling new groups of patients to be transplanted. The fine tuning of treatment protocols and the procurement of organs as well as improvement in surgical skills and techniques has played a central role in improving the long term graft survival. The discovery of histocompatibility antigens and identification of HLA specificity has also played a central role in first matching patients with donors and thereafter in risk determination of the transplantation. However, even in patients with 0 HLA mismatches rejections do occur. Comparing the results from 1984-1995 with today’s result Opelz and co-workers (2) found that rejection episodes during the first year after transplantation still occurs in almost 15% of the patients even though these patients are well matched (0 mismatches). Also in HLA identical siblings immunoreactivity can be high, leading to premature graft loss (4).

Hariharan and co-workers (1) published data from renal transplant recipients (n=93,934) in the US between 1988–1996 concluding that patients experiencing an acute rejection during the first year after transplantation had a reduced projected half-life of the transplant compared to patients not experiencing an acute rejection year 1 (Fig. 1). Hariharan et al, as well as others (5, 6, 7), found that acute rejection impairs long-term graft survival in renal transplant recipients. Rejections in

![Fig. 1: Adapted from reference 1. Acute rejection within the first year post-transplantation is a negative predictor of long term renal allograft survival. Projected half-life of grafts in patients (from 1988 to 1996) who did, respectively did not, experience an acute rejection episode within the first year.](image)

During the last 10 years the role of non-HLA antibodies has been widely accepted as contributors in post transplant rejections (8-17). The Collaborative Transplant Study (CTS) has investigated the role of non-HLA antibodies and even in HLA identical siblings Panel Reactive Antibody (PRA) activity is present and influences the long term survival of the graft (Fig. 2). Since donor specific HLA antibodies are probably not involved in rejection of these grafts the PRA indicates the degree of immunization which may be correlated with the presence of non-HLA antibodies. When the long-term results for kidney recipients with PRA were examined over 10 years of follow-up the influence of non-HLA-directed immunity was of similar magnitude to that of antibodies against HLA (4).

Recent studies have been published analyzing Anti Endothelial Cell Antibodies (AECA) in both renal (18) and heart transplanted (19) patients and these studies confirm previous findings (reviewed in Holgersson et al, reference 20) that AECA are strongly associated with poor outcome and increased number of rejections.

Although the specificities of non-HLA antibodies in organ rejection still remains to be defined, a number of non-HLA antibodies with relevance in organ transplantation and rejection have been shown to be AECA of different specificities (Fig. 3). Until recently it has not been possible to routinely screen for AECA and there has been a need for reproducible assays that can detect and quantify these antibodies (21).

Graft survival is affected by non-HLA immunity

![Fig. 2: Adapted from reference 2. The prevalence of PRA in HLA identical siblings and its correlation to graft survival.](image)
Endothelial cells – Targets of HLA and non-HLA antibodies

Importance of identifying patients at risk for rejection, that are not detected with standard crossmatching techniques.

- HLA class I & II are not the only antigens involved in rejection. Lymphocytes, used in standard crossmatch testing, do not express the whole range of antigens present on a transplanted organ.
- The donor endothelium is the first tissue that a patient’s blood encounters, and endothelial cells express relevant surface antigens that are targets in organ rejection. Antibodies against endothelial cells have been shown to be important for clinical outcome in transplantation.
- Endothelial precursor cells isolated with XM-ONE are more alike the graft tissue compared to lymphocytes, used in conventional crossmatches.
- Previously it has not been possible to routinely test for the presence of donor specific anti-endothelial cell antibodies (AECA).
- XM-ONE® is an endothelial cell based crossmatch test which enables rapid detection of AECA as well as antibodies against HLA class I and II antigens.

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<th>SPECIFICITIES OF NON-HLA ANTIBODIES WITH RELEVANCE IN ORGAN TRANSPLANTATION</th>
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AR: Acute rejections; HAR: hyperacute rejections; EGL: early graft loss; CR: chronic rejection; VR: vascular rejection.

XM-ONE® – A Tool for Enhanced Risk Assessment

A large proportion of transplant patients experience at least one rejection episode despite receiving a blood group matched organ and an extensive pre-transplantation testing regime. Apart from the increased risk of irreversible organ damage, hyperacute or accelerated acute rejections in some cases result in actual graft loss. Preformed antibodies against non-HLA antigens expressed on endothelial cells are one important cause for the development of hyperacute or early acute rejections in patients whose lymphocyte crossmatch test is negative. AECA antibodies are correlated to higher incidence and severity of acute rejections.

Testing for AECA antibodies in organ transplant recipients gives further information on immunoreactivity in the patient leading to improved risk awareness.

XM-ONE® positive – an increased risk for rejections
XM-ONE® testing provides additional information to a traditional lymphocyte crossmatch. The test identifies patients at increased risk of rejection due to donor specific AECA.

Compared to XM-ONE® negative patients, XM-ONE® positive patients:
- experience more early rejection episodes
- experience more severe rejection episodes
- have an increased incidence of C4d positive deposit / antibody mediated rejections

Fig. 3: Adapted from reference 22. Non-HLA antibodies associated with graft rejections.
The main objectives of the international multicenter study\(^a\) were to determine the frequency of XM-ONE® positive patients in the transplanted population and investigate the difference in occurrence of rejection episodes after kidney transplantation between patients with a positive and a negative XM-ONE® result.

Patient enrollment was based on acceptance for transplantation as determined by conventional cytotoxic and/or flow cytometry based lymphocyte crossmatch results. A total of 147 patients were evaluated in six transplant centres, both in the US and in Sweden.

- **XM-ONE® positive patients experienced more early rejections.**
  In the study 24 % (35/147) of the patients had a positive XM-ONE® test result. These patients had a significantly higher risk for rejection episodes than those with a negative XM-ONE®. All acute rejections in the XM-ONE® positive patients occurred within the first three weeks of the study (46%, 16/35, Fig. 4) while the XM-ONE® negative patients had a lower incidence of rejections and only 5% of the rejections occurred within the first three weeks (total 12%, 13/112 entire study period).

- **XM-ONE® positive patients developed more severe types of rejections.**
  All antibody mediated rejections occurred in the XM-ONE® positive group (Fig. 5). Only one graft loss occurred in the study and this patient had a positive XM-ONE® test.

**Conclusion**
The study showed that the XM-ONE® test can detect an antibody population not possible to detect with lymphocyte crossmatch tests that is strongly associated with rejection episodes and reduced kidney function after transplantation. Thus the XM-ONE® test can identify patients at risk of antibody-mediated rejections.
**XM-ONE® – Instructions for Use**

**Intended Use:** XM-ONE® is an in vitro diagnostic kit which is used for the isolation of endothelial precursor cells expressing endothelial and monocyte markers and preparation of samples for analysis of IgM and IgG antibodies specific for these cells. The kit also contains reagents that makes it possible for the user to optionally perform lymphocyte crossmatches in conjunction with the assay.

**Precaution:** For In Vitro Diagnostic Use. This product is intended to be used by healthcare professionals only. In addition the person responsible for analyzing the result should be experienced in flow cytometry. Once used, all materials should be disposed of as biohazardous waste. Handle all biological samples as potential infectious.

**Storage:** Magnetic beads and secondary antibodies should always be stored at a temperature between +2°C and +8°C. Vacutainer® CPT Tubes and siliconized glass tubes can be stored at 18°C–25°C temperature. Vacutainer® CPT Tubes should be at 18°C–25°C temperature when used. All reagents in the kit should be kept away from direct sunlight. The label on the kit displays the expiry date. All components of the kit should be used before this date. The secondary antibodies shall, after opening, be used within two months.

**Kit contents and reagents:** This kit contains components for 4 tests; this includes 4 vials of bead suspension, 4 siliconized glass tubes, 1 vial 400 μl of Anti IgM, 1 vial 400 μl of Anti IgG, 1 vial of 800 μl anti-CD3, 1 vial of 800 μl anti-CD19, 16 BD Vacutainer® Heparin CPT tubes and 1 instruction for use.

**Analysis of XM-ONE® results using Flow Cytometry**

**Endothelial cell crossmatch (Gate R1)**

**T-cell crossmatch (Gate R2 and CD3 positive)**

**B-cell crossmatch (Gate R2 and CD19 positive)**

XM-ONE® provides optimized reagents for flow cytometric detection of antibodies that can bind to endothelial precursor cells (Gate R1), enriched for with XM-ONE®, as well as lymphocytes (Gate R2).

Secondary reagents are coupled to RTIC which can be detected by the FL1 channel. Cells showing a positive result show an FL1 shift compared to cells incubated with negative control serum. Note, for B cell crossmatching with XM-ONE®, the number of B cells are fewer compared to the number of isolated EPCs and T cells.

Validation tests of lymphocyte crossmatching using XM-ONE® and standard T- and B-cell crossmatch method show that the channels shifts highly correlate between XM-ONE® and the standard lymphocyte crossmatch techniques.25

*Now including CD3 and CD19 antibodies for simultaneous T- and B-cell crossmatching.