ISSN (Print): 0974-6846
ISSN (Online): 0974-5645
Indian Journal of Science and Technology, *Vol 11(25), DOI: 10.17485/ijst/2018/v11i25/120321, July 2018*

A Comparative Study in Using Solid Phase Assays as Technics in a Screening of Anti-HLA Antibodies

Ghizlane Saadane[1]*, Mounia Oudghiri[2], Abdellah Naya[2], Benyounes Ramdani[3] and Siham Bennani[4]

[1]Faculty of Sciences Ain Chock, University Hassan II km 7 Route d'El Jadida and Pasteur Institute of Morocco 1, Place Louis Pasteur; saadane.ghizlane@gmail.com
[2]Faculty of sciences Ain Chock, university Hassan II km7 route d'El jadida, Morocco; mouniaoudghiri@gmail.com, anaya@mabiotech.com
[3]2IbnRochd University hospital, Lahcen EL Arjounstreet Casablanca, Morocco; Ramdanibenyounes@gmail.com
[4]Pasteur Institute of Morocco 1, Place Louis Pasteur; siham.bennani@yahoo.fr

Abstract

Objectives: Introduction of solid phase assays for HLA antibodies detection led to application of new algorithms for the immunological monitoring of patients in renal transplantation and prevented many cases of hum oral rejection. **Methods:** In this work, we made a single observation of a comparative study between ELISA and LUMINEX® on 60 patient's sera prior to start using in routine LUMINEX® at the two compatibility department of Pasteur Institute Casablanca, Morocco. Data were analyzed following manufacturer recommendations. **Finding:** Concordance rates between the results for individual HLA Class I and Class II antigens measured by both techniques were found to be 73, 3%. Whereas discrepancies were around 25%. The use of sensitive and specific techniques in transplantation is an important parameter which could improve this field. **Improvement:** Following this study, significant difference between the tests was found, however LUMINEX detect HLA antibodies with higher sensitivity, which can provide additional information for the graft outcome.

Keywords: Anti-HLA Antibodies, Kidney Transplantation, ELISA, LUMINEX

1. Introduction

Anti HLA antibodies, are formed by the immune system, they occur following multiple pregnancies, blood transfusions, and previous transplantation. Since it has been established that they're the most common physiological obstacle to the success of kidney transplantation, they became the main objective transplantation field[1].

To evaluate the immunological status of patients, a screening for reliable, sensitive and specific techniques is important. It enables to monitor the patient's outcome and establishes a targeted treatment in case of rejection.

The Complement Dependent Cytotoxicity test (CDC) was described for the first time[2] and used for decades as the gold standard for anti HLA antibodies screening. In the middle of 90's, the introduction of solid phase assays has allowed a better and more specific detection of antibodies; such as the Enzyme Linked Immunosorbent assay method where antigen are fixed in TERASAKI plate, and the LUMINEX technical where antigen are coated in animusofluorescent beads. The latter technical was recently introduced at the two compatibility unit at Pasteur Institute of morocco, whence the importance to lead a comparative study between both ELISA and LUMINEX for screening of anti HLA antibodies.

In this study, sera of 60 patients were tested in order to confirm the previous results of anti HLA screening obtained while using ELISA, and to evaluate the Sensitivity and specificity of LUMINEX technical. We provide an additional data regarding the efficiency of solid phase assays which are an important asset to the clinician in kidney transplantation.

*Author for correspondence

2. Methods

2.1 Patient Population

This is a single center study of 60 sera of patients (41, 64% women/58, 35% men) from transplant center in IbnRochd University hospital Casablanca, Morocco. The HLA Followed up was done at histocompatibility Unit of Pasteur Institute of morocco between September 2011 and December 2013.

46 patients were awaiting kidney transplantation, and 14 sera was one year post-transplantation. 68% had previous blood transfusions. All the patients were CDC Cross match test negative.

Blood was initially collected from patients in a dry tube and was directly centrifuged and allowed to settle. Sera were then retrieved and stored at -20°C until needed. All the techniques were performed following the manufacturer's instruction.

2.2.1 Inclusion and Exclusion Criteria

All the patients that were CDC negative and previously screened by ELISA were included in the study. All patients in post-transplant had a good follow-up. Sera for which results by ELISA were completely confirmed were excluded. Patients with incomplete clinical information as previous immunization, demographic data and who had over than 2 blood transfusions were excluded.

2.2 Enzyme Linked Immunoabsorbantassay

Anti HLA antibodies screening was conducted at first by ELISA, LAT-mixed Class I/II (LAT™, One lambda, Canoga Park, CA).

The test provides pre-calibrated ELISA reagents for IGG antibody detection for HLA Class I or Class II antigen in human serum. Defined amounts of affinity purified HLA antigens are present in different wells of a TerasakiTray; the antibody specific binding from the test sample with any of these antigens is detected by a subsequent incubation with alkaline phosphates-conjugated antibody that recognizes only human IGG. Reaction quantitative measurement is obtained by spectrophotometric determination by following the addition of the appropriate substrate of the enzyme. HLA fusion is used for results interpretation. Test validation requires, a positive control DO that must be superior or equal to 5 times the negative control and at least equal to 50% of the positive control IgG, and a ratio of net OD of both positive and negative controls than 8; Net OD is represented by the average value of the background reaction noise (OD measured blank wells = white reaction). The assay cut-off (S) is determined by multiplying the average net OD of the positive control HLA by 0.20, and the gray zone is between 1 and 1.25 times the threshold. Sera were considered positive when the net OD value is greater than the upper limit of the gray area (1.25 × S) and as negative when the net OD value is inferior to the threshold value.

2.3 LUMINEX Technology for Anti HLA Screening and Identification

In the Second step, the screening and identification were realized by LIFECODES life screen LMX Deluxe, and LIFECODES LMI/ID LM2Q for identification. The kits are commercialized by GenProbe (San Diego, CA), According to the manufacturer's instructions; the data were analyzed by using Match IT (1.18.1.1) software.

The test involves a first incubation of beads with the patient's sera. HLA antibodies that react with beads expressing corresponding HLA molecule are incubated with a secondary antibody conjugated to a phycoerythrin (PE) anti human IGG. The readout is then performed using a LUMINEX fluorocytometer.

The signal intensity from each bead is compared to the signal intensity of negative control sera and negative control beads included in the bead preparation to determine if the bead is positive or negative for bound alloantibody.

To determine if an individual HLA bead is positive, the individual bead MFI is divided by the MFI for each Negative Control Bead (CON1, CON2 and CON3). From these quotients, the Background Adjustment Factor (BAF) is subtracted for the appropriate bead/CON combination.

The BAF is a pre-determined as the MFI ratio for each bead/CON combination to compensate for background noise due to bead variation. For PROBE I-01 and PROBE II-01, a positive value for any one of previous calculated measurements indicates a positive bead reaction. For all remaining beads, a positive value for any two of the calculations indicates a positive bead reaction. A negative value for all three calculations indicates a negative bead reaction.

A sample is considered as positive for class I HLA-specific antibodies if at least one of the seven (7) class I HLA beads is positive. And a sample is considered as positive for class II HLA-specific antibodies if at least one of the five (5) class II HLA beads is positive. And a sample is considered

as negative for HLA-specific IgG antibodies if all the HLA beads are found to be negative.

Statistical analysis was carried out using IBM SPSS Statistics software, version 22 for Windows XP, and fisher's exact test was used to calculate results significance. A p-value less than 0.05 were considered significant.

3. Results

3.1 Screening Results: ELISA vs LUMINEX

Results obtained for 60 sera patients by ELISA and LUMINEX were analyzed, and a threeshould supplier was used for interpretation.

Concordance and discrepancies results for HLA Class I and HLA Class II are reported respectively in Table 1 and 2.

Concordance was observed for 44 sera (73, 3%), where 34 were ELISA Negative- LUMINEX Negative, and 10 sera was POSITIVE using both ELISA and LUMINEX. While discrepancy was obtained for 16 sera patients (26, 6%), where 10 sera were ELISA negative/LUMINEX positive, and 6 sera ELISA positive- LUMINEX negative. (p = 0.006) (Table 1).

Table 1. Screened sera results in HLA Class I using ELISA/LUMINEX

	LUMINEX NEGATIVE	LUMINEX POSITIVE	TOTAL
ELISA NEGATIVE	34	10	44
ELISA POSITIVE	6	10	16
TOTAL	40	20	60

In HLA class II, the concordance was obtained for 44 sera (73, 3%), where 35 sera were ELISA negative– LUMINEX negative, and 9 sera positive for both ELISA and LUMINEX.

In the other hand, discrepancy was observed for 16 sera (26, 6%), where 6 sera were ELISA positive-LUMINEX negative, and 10 sera ELISA negative- LUMINEX positive. (p = 0.0105) (Table 2).

Table 2. Screened sera results in HLA Class II using ELISA / LUMINEX

	LUMINEX NEGATIVE	LUMINEX POSITIVE	TOTAL
ELISA NEGATIVE	35	10	45
ELISA POSITIVE	6	9	15
TOTAL	42	18	60

FP: False Positive / FN: False Negative / TP: True Positive / TN: True Negative

3.2 Identification results

In order to set a complementary comparison between ELISA and LUMINEX, an additional test was performed by identification. Sera choose where those with a screening result not clearly determined in screening by LUMINEX. Sera were considered false positive, or false negative if at least one results was discordant.

In class I, 9 identifications were performed, and 1 sera false positive, 1 sera false negative (Table 3) were obtained, while in class II only one sera was false positive (Table 4).

Sensitivity and specificity were calculated by integrating the results of identification which were made to resolve discrepancies observed during initial analysis. The sensitivity, and specificity rates of LUMINEX tests, was respectively 58, 33% and 75, 82%.

Table 3. Identifications results obtained using LUMINEX for discrepancies sera in HLA Class I

N° of sera	ELISA Screening	LUMINEX Screening	LUMINEX Identification	Results
9	NEG	NEG	POS	FP
13	POS	POS	POS	TP
22	POS	NEG	NEG	FP
25	POS	POS	POS	TP
44	POS	NEG	POS	FN
47	NEG	NEG	POS	FP
51	POS	POS	POS	TP
53	POS	POS	POS	TP
55	NEG	POS	POS	FN

Table 4. Identifications results obtained using LUMINEX for discrepancies sera in HLA class II

N° of sera	ELISA Screening	LUMINEX Screening	LUMINEX Identification	Results
1	NEG	POS	POS	FN
9	NEG	POS	NEG	FP
10	POS	POS	POS	TP

N° of sera	ELISA Screening	LUMINEX Screening	LUMINEX Identification	Results
13	POS	POS	POS	TP
22	POS	NEG	NEG	FP
25	POS	POS	POS	TP
44	POS	NEG	NEG	FP
47	NEG	POS	POS	FN
51	POS	POS	POS	TP
55	POS	POS	POS	TP

4. Discussion

Recipients whom are sensitized to HLA antigen after immunizing events represent one of the most critical issues in organ transplantation, increasing the frequency of rejection episodes[3]. It is therefore important to monitor patient in pre-transplant period to reduce the risk of acute rejection, to allocate adequate immunosuppressant and improve post-transplant monitoring[4]. Over the years technics of screening and identification have had a crucial role in granting a compatible organ. Initially, microlymphocytotoxic test was used for the determination of anti-HLA antibodies using a panel of T lymphocytes with a large representation of different Class I antigens, sera reacting with the majority or the entire panel are those highly immunized[5]. Even the test was used for decades as the gold standard, number of technical problems persisted, as assay depend on the viability of the donor cells and in the case of deceased donors optimal viability is not always maintained, the test also detect IGG, IGM as well as auto-antibodies[6].

Introduction thereafter of solid phase antibody detection assays such as ELISA and LUMINEX have helped to gradually replace the CDC, and enabled for several laboratories through number of studies to confirm their sensitivity and efficiency[7–9]. Our study was performed on 60 patient's sera in pre- and post-transplant. Sera were initially screened by ELISA, where in Class I, 56.6% of the sera were negative and 44.4% positive. The 60 sera were then screened by using LUMINEX technology. We thus obtained a concordance in class I and II of 73% ($p = 0.001$). These results are statistically significant. Results obtained by LUMINEX technology have cleared ambiguities in cases screened by ELISA where the values of PRA were in grey area. However, discrepancies obtained between both techniques as well as between different levels of screening and identification for some sera with LUMINEX could be explain by several technical parameters. On the one hand the use of reagents from two different companies, with different principles and protocols[10], on the other hand the nature and expression of antigen as well as secondary reagents and the readout used[11,12]. Therefore, bead assay represent the most sensitive method for HLA antibodies screening and identification and it was widely discussed. Results obtained showed a higher sensitivity and specificity. Results were similar to those reported[13], another study has shown better sensitivity and specificity in class I and class II for comparing to ELISA assay[14]. Furthermore a higher sensitivity has also been reported for LUMINEX technique compared to ELISA[15]. As the main issue with the CDC assay was its sensitivity, solid phase assays provide better results. In fact, ELISA is more sensitive than CDC in detecting HLA antibodies, but has the potential drawback of not distinguishing between complement fixing and non-complement fixing antibodies. This issue was superseded by introduction of bead assays technology. In addition, to previously grafted patients and optimize the percentage of success of transplantation it is important to use a sensitive and specific technics since the use of different methods for detection of identification of HLA antibodies may lead inevitably to discrepancies[16]. The additional sensitivity enabled thus the detection of antibodies which was not detectable by CDC and ELISA and improved the success rate of transplantation. Lastly, as the main issue with CDC is its sensitivity, ELISA had initially been used for years in our laboratory but was unable to clearly define HLA specificities in such cases with a high PRA (up to 70%). Introduction of LUMINEX assay improved screening and identification results even for poorly sensitized sera; it is also provide an appropriate platform to study impact of different is types of antibodies on the outcome of transplantation. . It was thus used as the reference technical to identify the exact HLA profile of patients. That being so, CDC test is still functional as final test of pre-transplant compatibility.

5. Conclusion

The presence of reactive antibodies and their clinical impact are a real public health dimension in the prevalence of all immunization of patients. The use of sensitive and specific techniques should take into account the limitations of each, while strict interpretation takes into account the balance of patient histocompatibility and immunological status.

6. Acknowledgments

We thank Mr AKLI Bouaziz who made a critical revision of our manuscript and for his scientific support during data interpretation.

7. Limitations of study

One of the limitations of our study is that sera were not tested twice using assays. If the tests were performed twice, it might confirm if additional difference between assays occurs, and it might show if there was any change in results. Furthermore, there was no additional demographic data provided for sera tested.

8. Declarations

Abbreviations: HLA: Human Leukocyte Antigen / DSA: Donor Specific Antibodies / MFI: Median Fluorescence Intensity

Author's Contributions: GS carried out the immunoassays, statistical analysis and redaction of manuscript. OM participated in the study design, and critical revision of manuscript. AN made contribution in the study design and critical revisions of manuscript. BS participated in the data analysis, interpretation, and conception of the study design. BR gives the final approval for manuscript submissions. All authors read and approve the final manuscript

Competing Interest: The authors declare that they have no competing interests.

Availability of Data and Materials: Data will not be shared, because all the data for this study are contained in the present document.

Consent to Publish: Not Applicable

Consent to Participate: Not applicable

Ethics: Not applicable

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors

9. References

1. Glotz D. Transplantation rénale de patients immunisés dans le système HLA et immunoglobulines intraveineuses. In : Lavoisier médecine sciences editors. Paris: Actualités néphrologiques Jean Hamburger, Publication Company; 2000. p. 1–11.
2. Patel R, Terasaki PI. Significance of the positive cross match test in kidney transplantation. Nen Gland Journal Medicine. 1969Apr; 280(14):735–9. Crossref. PMid:4886455
3. Sumitran S. Importance Clinique des anticorps HLA et non HLA en allotransplantation rénale. In: Medecine Sciences Flammarion. Paris: Actualités Néphrologiques Jean Hamburger; 2000. p. 1–9.
4. Lefaucheur C, Nochy D, Glotz D. Rejet aigu médié par anticorps. Néphrologie et thérapeutique. 2008 Oct; 4(3):188–91. Crossref.
5. Legendre C. La transplantation rénale. Paris: Lavoisier Médecine Publication Company; 2012. p. 1–942.
6. Tait BD. Detection of HLA antibodies in organ transplant recipients - Triumphs and challenges of the solid phase bead assay. Frontiers in immunology.2016 Dec, 7 (570), pp. 1-11. Crossref.
7. Colombo MB, Haworth SR, Poli F, Nocco A, Puglisi G. Luminex technology for anti HLA antibody screening: Evaluation of performance and of impact on laboratory routine. Cytometry B Clincytom. 2007 Nov; 72(6):465–71. Crossref. PMid:17397064
8. Mehrota S, Sharma RK, Mayya M, Gupta A, Prasad N, Kaul A, Bhadauria DS. Pre transplant PRA (Panel Reactive Antibody) and DSA (Donor Specific antibody) screening and outcome after renal transplantation. Indian Journal of Transplantation. 2015; 9(1):7–12. Crossref.
9. Heinemann FM. HLA genotyping and antibody characterization using the LUMINEX multiplex technology. Transfusion Medicine and Hemotherapy. 2009Aug; 36(4):273–8. Crossref. PMid:21049078 PMCid:PMC2941832
10. Israeli M, Pollack MS, Shaut CA, Halpin A. Concordance and discordance in anti HLA antibody testing. Transfusion Plantimmunology. 2015 Jan; 32(1):1–8. Crossref.
11. Bray RA. Clinical CYTOMETRY and progress in HLA antibody detection. Methods in Cell Biology. 2011; 103:285–310. Crossref. PMid:21722808
12. Gebel HM. Technical aspects of HLA typing. Current Opinion in Organ Transplantation. 2013 Aug; 18(4):455–62. PMid:23838651
13. Goggins R. Luminex lab screen mixed as a qualitative antibody-screening assay. Human Immunologies. 2002 Oct; 63(10):1–104.
14. Souhaila A, Dubois V, Benhalima M. La technologie de phase solide pour la détection des anticorps HLA ELISA versus LUMINEX: Les défis de l'interprétation. Revue Francophone Deslaboratoires. 2012 Jul; 444:79–84.
15. Eng HS, Lefell MS. Histocompatibility testing after fifty years of transplantation. Journal of Immunological Methods. 2011 Jun; 369(1-2):1–21. Crossref. PMid:21530531
16. Jung S, Oh EJ, Yang CW, Ahn WS, Kim Y, Park YJ and Han K. Comparative evaluation of ELISA and LUMINEX panel reactive antibody assays for HLA alloantibody screening. Korean Journal Lab Medicine. 2009; 29(5):473–80. Crossref. PMid:19893358