Comparing MFI and MCI in single antigen HLA antibody detection on alternative platforms: Luminex vs HISTO SPOT® microarrays

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Introduction

The detection of HLA antibodies is critical for the management of organ transplantation. The Luminex platform has been widely used for this purpose, providing reliable results through Mean Fluorescence Intensity (MFI) measurements. However, new technologies, such as the HISTO SPOT® microarrays, are emerging as potential alternatives, utilizing Mean Color Intensity (MCI) for antibody detection.

This study aims to directly compare the performance of the Luminex and HISTO SPOT® platforms in detecting single antigen HLA antibodies. A key objective is to establish a comparison factor between MFI and MCI, enabling a standardized assessment of these two technologies. Understanding the advantages and limitations of each platform will help in optimizing diagnostic strategies and improving patient outcomes.

Methods

Comparing Different Methods and Devices

Five sera from the German quality exchange program (INSTAND February 2024) were used to compare different methods and devices for HLA antibody detection. The following methods were employed:

- **Luminex Methods**:
  - LIFECODES LifeScreen LSA Class I Luminex assay (Werfen) [LSA]
  - LABScreen Single Antigen Class I Luminex assay (ThermoFisher/ One Lambda) [OLI]
- **HISTO SPOT® Method:**
  - HISTO SPOT® HLA AB ID Class I assay (BAG Diagnostics) [BAG]

The comparisons involved testing on different devices and utilizing varying reagent approaches for LSA, including a protocol variation where 75% of the reagents were used according to HLA. 2016 Sep;88(3):110-9. The Luminex assays were performed on multiple devices (Luminex 200, FlexMAP 3D), while the HISTO SPOT® assays were conducted using both the MR.SPOT® and MR.SPOT® 2.0 analyzers, resulting in six distinct test groups.

Standard Curve Creation for Method Comparison

To compare the results of different methods for identifying single antigen HLA antibodies, a normalization factor is necessary. A standard curve was generated using the monoclonal antibody W6/32. Various concentrations of mAB W6/32 were tested with the LIFECODES LifeScreen LSA Class I Luminex assay (Werfen) and the HISTO SPOT® HLA AB ID Class I assay (BAG Diagnostics). For each concentration, the mean and standard deviation (SD) of the MFI and MCI values were calculated to create the standard curve.

Results

Comparing Different Methods and Devices

Only the positive HLA class I antibody specificities according to INSTAND were included in the analysis to compare the data. Figure 1 shows the MFI/MCI results of the five samples for each of the different assays. There is a large variability between MFI and MCI values obtained from different assays and different analyzers. Statistical analysis using paired T-tests showed that the differences between the different assays and analyzers were statistically significant (Figure 2).

Standard Curve Creation for Method Comparison

Due to the significant differences in absolute MFI values obtained, a dilution curve of the mAB W6/32 was used to establish a normalization factor for the MCI and the MFI based on the MFI/MCI values at IC50 (calculated using GraphPad Prism 10.2.2 Software). From these dilution curves (Figure 3), the following values for the IC50 and the MFI/MCI values at IC50 are calculated for these specific two instruments:

- **HISTO SPOT® HLA AB:** IC50 = 0.008 mg/ml, MCI(IC50) = 8681
- **LIFECODES LSA Class I:** IC50 = 0.297 mg/ml, MFI(IC50) = 5548

From the MFI/MCI values at IC50, a transformation factor of 0.64 is derived to predict MFI values from the MCI values measured with the HISTO SPOT® HLA AB test.

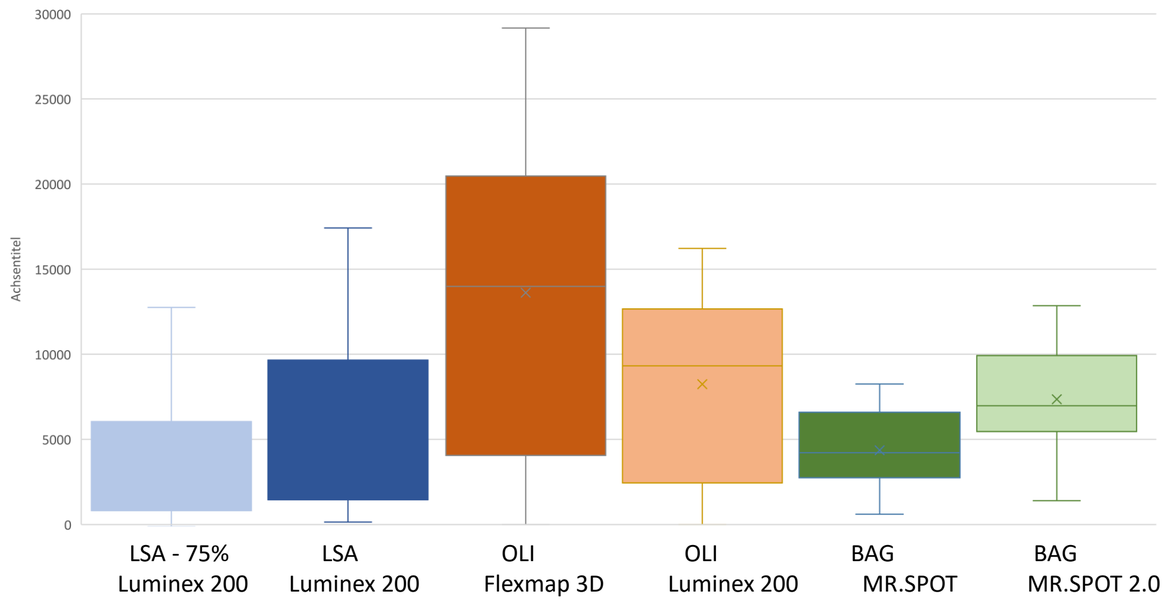

Fig 1. Boxplots of the MFI/MCI Results for the Different Assays

	LSA - 75% Luminex 200	LSA - Luminex 200	OLI Flexmap 3D	OLI - Luminex 200	BAG - MR.SPOT	BAG - MR.SPOT 2.0
LSA - 75% Luminex 200	x	p < 0.0001	p < 0.0001	p < 0.0001	p = 0.0214	p < 0.0001
LSA - Luminex 200	x	x	p < 0.0001	p < 0.0001	p = 0.0004	p < 0.0001
OLI - Luminex Flexmap 3D	x	x	x	p < 0.0001	p < 0.0001	p < 0.0001
OLI - Luminex 200	x	x	x	x	p < 0.0001	p < 0.0001
BAG - MR.SPOT	x	x	x	x	x	p < 0.0001
BAG - MR.SPOT 2.0	x	x	x	x	x	x

Fig 2. Statistical Analysis Using the Paired T-Test

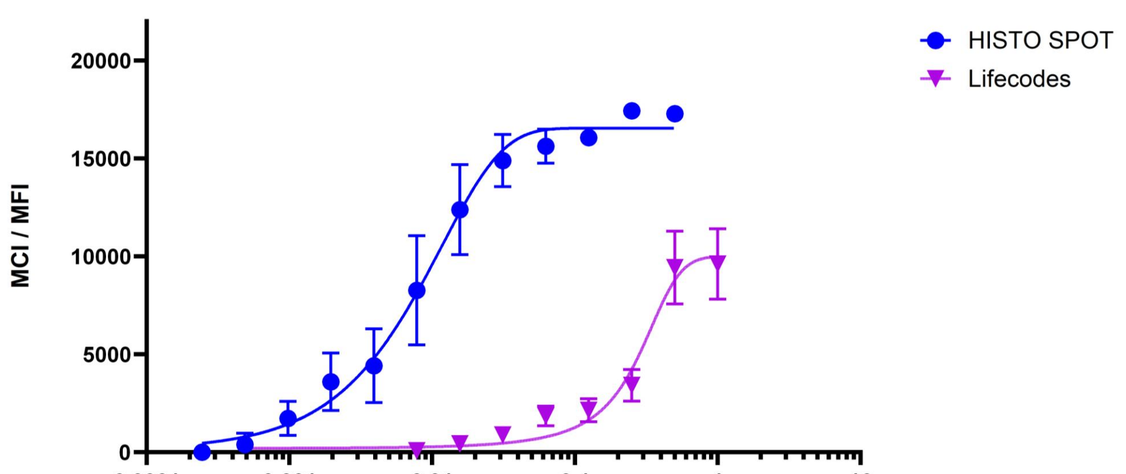

Fig. 3: MCI/MFI for Different Dilutions of W6/32 mAB with Antigens in HISTO SPOT® HLA AB Class I and LIFECODES LifeScreen LSA Class I on Luminex 200

Conclusion

Both Luminex and HISTO SPOT® microarrays are effective in detecting HLA antibodies, with a strong correlation between MFI and MCI values. The variability of MFI and MCI values obtained by assays from different manufacturers indicates that absolute values are not a reliable measure of antibody strength or clinical relevance. Reporting the pattern of positive reactions is more useful than absolute values. MCI values for positive reactions are within the variance of MFI values and can be reported as equivalents. If absolute values are reported, they should be standardized, e.g., by a dilution curve of a monoclonal antibody. The cost-effectiveness and comparable performance of the HISTO SPOT® microarrays make them a viable alternative to the Luminex platform. Future studies should focus on larger cohorts and real-world clinical settings to further validate these findings.

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