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Assessing the Clinical Utility of the HISTO SPOT® HLA AB Detection System in Clinical Histocompatibility Laboratories

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INTRODUCTION

Solid-phase Luminex® technology is the current standard for HLA antibody (Ab) testing but is prone to non-specific and spurious reactivities. The HISTO SPOT® HLA AB assay (BAG Diagnostics) is a novel, fully automated, ELISA-based method using single antigen (SA) chip arrays, reporting results as Mean Colour Intensity (MCI)—an analog to the Mean Fluorescence Intensity (MFI) used by Luminex.

The aim of the study was to evaluate the performance of HISTO SPOT® (HS) versus Luminex-based assays (LABScreen®, LS) in terms of:

- Concordance of positive/negative results
- Correlation between MCI (HS) and MFI (LS)

Given methodological differences, complete concordance was neither expected nor considered necessary.

MATERIAL AND METHODS

- **Samples:** 36 patient sera previously tested with LS; 4 also tested with LIFECODES® LSA.
- **Assays:**
  - HS performed per manufacturer's IFU (dilutions: 1:10 for class I, 1:80 for class II).
  - Results analyzed with HISTO MATCH AB software which helps assign positive and negative reactions and reports intensities as MCI values (Figure 1).

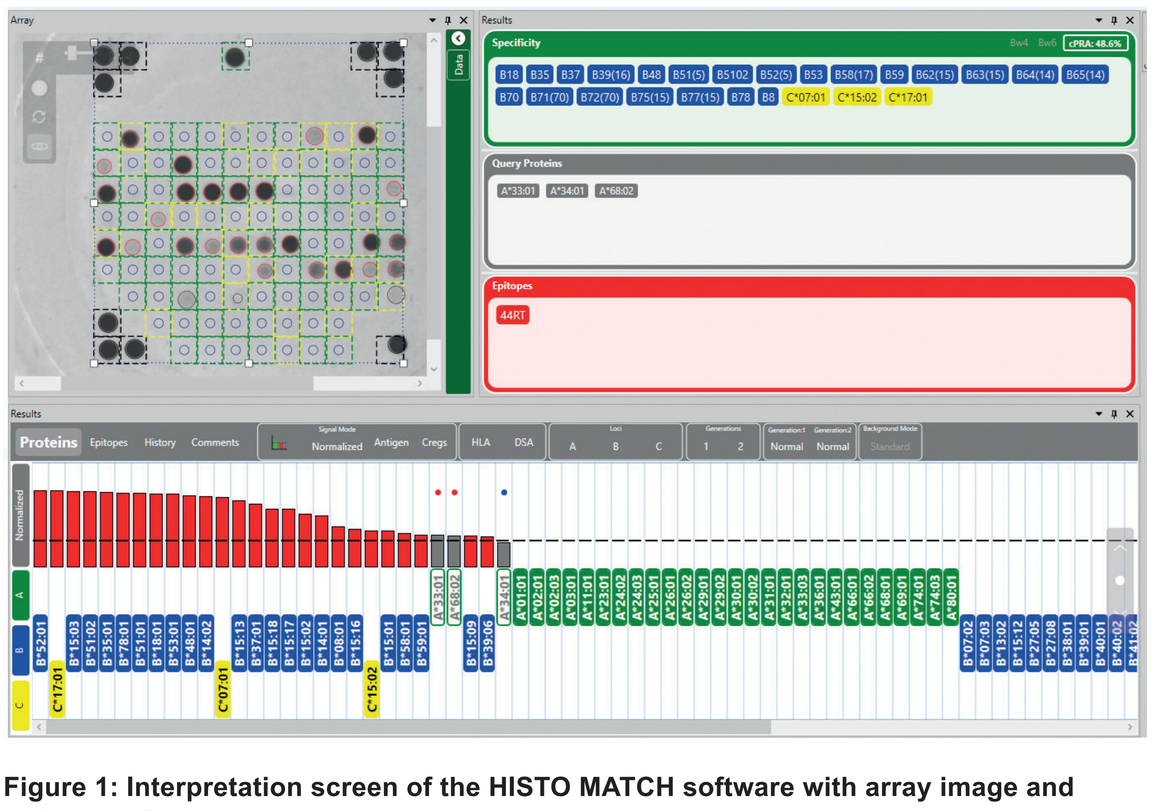

Figure 1: Interpretation screen of the HISTO MATCH software with array image and editing tools

- **Antigen panels:** 86 HLA class I, 69 HLA class II shared alleles (including specific α/β chain combinations).
- **Number of antigen-antibody reactions assessed:**
  - 3052 class I
  - 2386 class II
- **Exclusion:** Sera with broad, non-specific reactivity patterns such as pan-HLA class II, pan-HLA-DR, or pan-HLA-B patterns—on either platform.

RESULTS AND DISCUSSION

A) Concordance of results

Overall concordance of positive and negative results using a cutoff of MFI > 1000 for LS and the user edited assignments based on visibility of spots for HS was

- 92% for class I
- 94% for class II

Figure 2 shows the number of antigen-antibody interactions that were positive or negative for both tests and the number of interactions where only one of the two tests was positive. It shows that in class I there were more reactions that were only positive with the HS test and in class II there were more with LS test. For most of these discrepant reactions it could not be decided if they were correct or false positive. Using the LIFECODES® LSA test as a third test 49 of the reactions only seen with LS and 20 of the reactions only seen by HS were shown to be false positive.

Figure 2: Number of antigen-antibody interactions for different outcomes (comparison of 36 sera with HS (HISTO SPOT) and LS (LAbScreen), 4 sera tested with LIFECODES (concordant in class II with LS and HS))

Concordance stratified for negative, weak, moderate and strong reactions in the LS test is shown in Figure 3.

The concordance was highest for negative and strong reactions. As expected weak and moderate reactions showed a lower concordance.

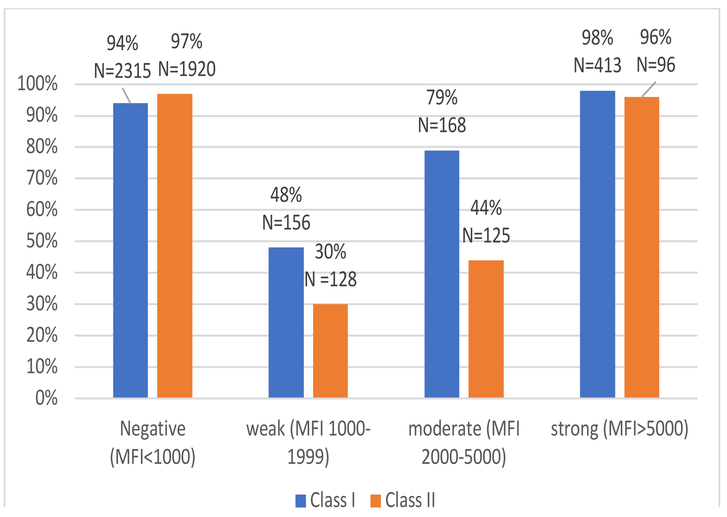

Figure 3: Concordance of results between LS and HS test stratified for negative weak, moderate and strong reactions in the LS test

B) Correlation between MFI and MCI

The correlation between HS MCI and LS MFI for positive reactions followed a hyperbolic function with R2 of 0.60 (class I) and 0.61 (class II) (Figure 4).

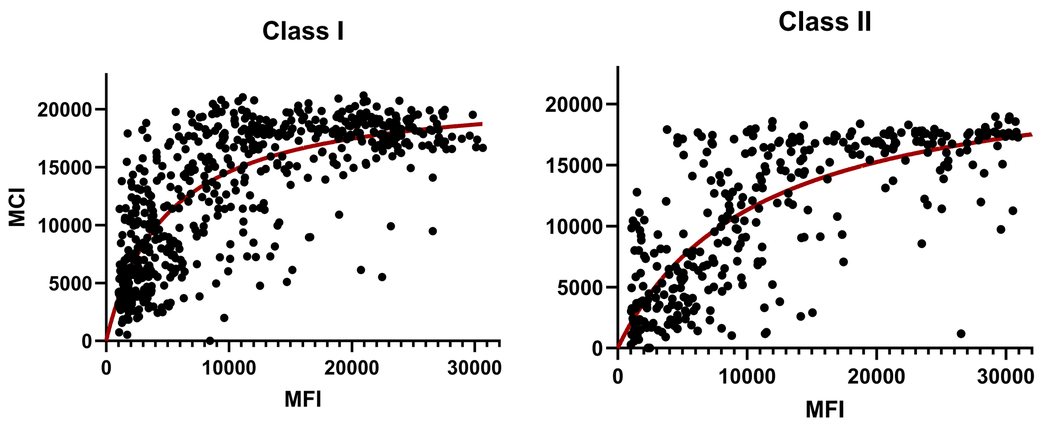

Figure 4: Correlation between MFI and MCI in the LS and the HS test

This corresponds to a hyperbolic correlation found between the MFIs from the LS and the LIFECODES LSA test found by Karahan et al. 2023.

CONCLUSION

Taken together, the HISTO SPOT HLA AB is a promising platform for HLA Ab detection. Our results corroborate previous studies demonstrating a decent agreement between HS and LS in HLA Ab assignments (Launhardt et al. 2021, Pedini et al. 2023, Emery et al. 2023, Duck et al. 2023). The fully automated, walkaway capability of HS is especially appealing for minimizing testing variability and workload in clinical HLA labs.

REFERENCES

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