Reproducibility of autoantibody titer assessment on HEp-2 cells in Brazilian Laboratories

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BACKGROUND

The HEp-2 indirect immunofluorescence assay (IFA) is the reference method for autoantibody screening, guiding antigen-specific testing and titer assessment. ANA titers ≥1:320 are strongly associated with systemic connective tissue diseases (1,2). Harmonization initiatives include the Brazilian Consensus (2000) (3) and the International Consensus on ANA Patterns (2014) (4). The third BCA (2009) recommended conjugate titer calibration and use of reference sera in quality assessments (5). This study assesses the reproducibility of HEp-2 titer determination in 67 Brazilian laboratories.

AIM

To evaluate the reproducibility of HEp-2 autoantibody titer determination among Brazilian clinical laboratories participating in an external quality assessment program.

METHODS

Fifteen serum samples, covering four defined reactivity ranges (negative, low, moderate, and high), were sent to 67 Brazilian clinical laboratories for HEp-2 autoantibody titer characterization. Results within one dilution above or one dilution below the nominal titer were considered acceptable. Concordance, discordance, overestimation, and underestimation indices were calculated based on the proportion of laboratories within or outside the acceptable range, with values representing the average of three evaluations per range. Sample distribution was coordinated by Controllab, a Brazilian EQA provider.

RESULTS

The mean concordance rate (CR) for negative patterns was 88.25%, with 11.8% of laboratories incorrectly assigning titers ≥1/80 (Discordance Index, DI). Accuracy was lowest for titers of 1/160 and 1/320, with CR values of 63.9% and 64.1%, respectively, and highest for 1/640 (72.4%) and >1/640 (93.2%). DI was lowest for negative samples (11.8%) and >1/640 (6.8%), but reached 27.6 - 36.1% for 1/160–1/640. Overestimation was most frequent for 1/160, whereas underestimation predominated for 1/320.

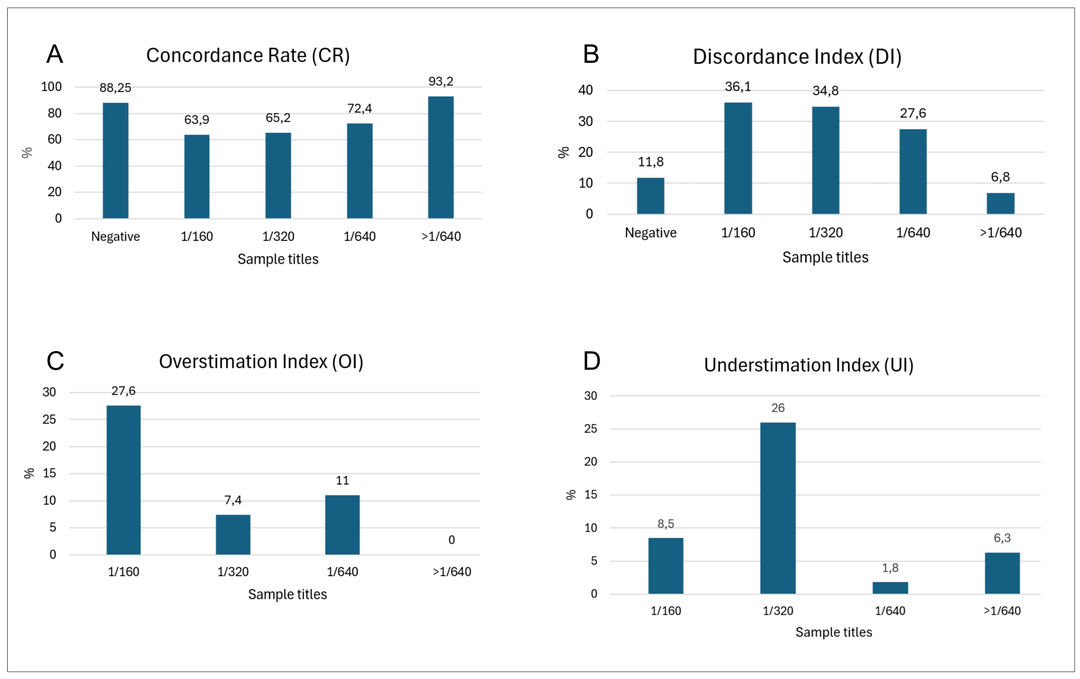
CONCLUSIONS

The study concluded that, while the identification of negative samples demonstrated high concordance (88.25%), inter-laboratory reproducibility varied significantly according to antibody titer. Low-titer samples (1:160 and 1:320) exhibited reduced concordance and higher discordance rates compared to high-titer samples (1:640 and >1:640), rendering them more susceptible to misclassification. Such discrepancies, particularly within the intermediate titer range, underscore the necessity for enhanced standardization to ensure greater consistency and reliability of results across laboratories.

Table 1: Results of the average of three assessments of samples at different concentration ranges, expressed as low, intermediate, and high titers:

SAMPLE	Concordance Rate (CR)	Discordance Index (DI)	Overestimation Index (OI)	Underestimation Index (UI)
Negative	88,25%	11,8%		
1/160	63,90%	36,10%	27,6%	8,50%
1/320	64,1%	35,9%	7,4%	26%
1/640	72,40%	27,6%	11,0%	1,8%
>1/640	93,2%	6,8%	0,0%	6,3%

Figure 1. Average of three assessments of samples across different concentration ranges (negative, low, intermediate, and high titers), showing the concordance rate, discordance index, overestimation index, and underestimation index for titer classification among 67 Brazilian clinical laboratories.



DISCLOSURE

The authors confirm that they don't have any conflict of interest to declare.

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