AUTOMATED IFA METHODS COMPARE WELL WITH ESTABLISHED MANUAL IFA SCREENING AND TITRATION FOR ANA HEP-2

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INTRODUCTION

The IIF using the HEp-2 cell substrate should be still considered the "gold standard" technique for determination of ANA. Automated systems have been developed to perform the complete pipetting of the slides and to analyze them by integrated microscopes.

Objective

This study was designed to compare two commercially available HEp-2 antinuclear antibody (ANA) by indirect immunofluorescent antibody assays (IIF). We compared the NOVA View® system (INOVA Diagnostics San Diego, CA USA) with the HELIOS® IFA Processor from AESKU.SYSTEMS (Wendelsheim, Germany) to assess their capability for screening, pattern recognition and titration of the samples.

MATERIALS AND METHODS

All in all, 198 samples were evaluated via direct microscopy by two expert physicians, providing a consensus decision of pattern and titer estimation. Pattern and titer from the instruments were compared with the declared pattern and titer and also from manually read slides.

RESULTS

1. Recognition of positive and negative pattern

Results of the two automated methods were in very good agreement in recognizing negative and positive samples. ***(TABLE 1)*** AESKUs® HELIOS® system reported 188 samples correctly as negative or positive, versus 187 reported by the NOVA View® System. The diagnostic sensitivity of the systems was 95.8% versus 96.7% for HELIOS® and NOVA View®, respectively. The systems exhibited a diagnostic specificity of 93.5% for the HELIOS® system and 91.0% for the NOVA VIEW®. The confidence intervals are indicated in the related tables.

2. Pattern recognition

120 positive sera were analyzed for the correct detection of both pattern and titer. The NOVA View® system detected the pattern correctly in 85 cases and also the HELIOS® system found 85 patterns that correlated with the target values. Including 78 negative sera, the HELIOS® detected 158/ 198 sera with the target pattern and the NOVA View® 156/198. ***(TABLE 2)***

3. Titer estimation

The HELIOS® system provided titer suggestions in 94 cases compared to 82 from the NOVA View® system. Taking into consideration the acceptable variability of </> one titer step the HELIOS® gave a correct estimated titer interpretation in 75 cases (NOVA View® 72 cases). Four samples were reported as false negative by the HELIOS® system and 4 as false positive (NOVA View® 3 false negative and 6 false positive samples).

Positive/Negative Agreement	*Expected Results/ Diagnosis*		
HELIOS®	*Pos*	*Neg*	*Total*
Pos	115	5	120
Neg	5	73	78
Total	120	78	198
Diagnostic Sensitivity	95% C.I.		
95.8%	90.6%	98.2%	
Diagnostic Specificity	95% C.I.		
93.5%	85.9%	97.2%	

Positive/Negative Agreement	*Expected Results/ Diagnosis*		
NOVA View®	*Pos*	*Neg*	*Total*
Pos	116	7	123
Neg	4	71	75
Total	120	78	198
Diagnostic Sensitivity	95% C.I.		
96,7%	91.7%	98.7%	
Diagnostic Specificity	95% C.I.		
91.0%	82.6%	95.6%	

TABLE 1
HELIOS® and NOVA View® versus expected results;
The false negative and false positive samples were all of low titer.

		HELIOS®	NOVA View®
All	198	**158**	**156**
Homogenous	42	33	36
Speckled	28	18	19
Centromer	13	8	11
Scl 70	3	3	3
Nucleolar (incl. PM-SCL)	13	10	8
Cytoplasmic	5	5	3
Cytoplasmic AMA like	5	5	4
Golgi	2	0	0
Cytoplasmic -Jo1	2	2	1
Rare Midbody – NOR 90 -FND	5	0	0
Nuclear membrane	2	1	0
Negative	78	73	71

TABLE 2 pattern recognition

DISCUSSION

This study is the first of its kind to include comparison of titer estimation of the HELIOS® system with that of the NOVA View® system from INOVA.
The diagnostic sensitivity of the systems was calculated as 95.8 versus 96.7% for HELIOS® and NOVA View®, respectively. Both systems exhibited good diagnostic specificity, 93.5% for the HELIOS® system and at 91.0 % for the NOVA View®. Summarized, the HELIOS® system detected 188 sera correctly in terms of positivity and negativity and the NOVA View® system 187 from 198. **The diagnostic efficiency was found to be 94.9% and 94.4% for HELIOS® versus NOVA View®.** The patterns suggested by both systems turned out to be reliable (HELIOS® 85/120 versus NOVA View® 85/120). With respect to the estimated endpoint titer it was observed that the NOVA View® system performed fewer determinations (83/120) while the HELIOS® calculated 96 titers. The number of titrations within the acceptable range of <>1 titer step from the recommended titer was comparable (HELIOS® 75/ NOVA View® 72). Here the HELIOS® system provides the possibility to align the titer according to the manual microscopy by a distinct factor setup.

CONCLUSIONS

Both systems resulted in an overall sensitivity of >95% (96.6% NOVA View® and 95.8% HELIOS®) and a specificity of 93.5 and 91% (AESKU® HELIOS® and NOVA View®, respectively). The correct recognition of the patterns showed a good quality in both systems. Both are suitable for fast and reliable detection of positivity/negativity due to their high sensitivity and will lead to a further increase of standardization in autoimmunity. The systems enable the user to utilize data banks including pictures and patterns of patients.