Automated Digital Microscopy Enhances the Reproducibility of Antinuclear Antibody Testing

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ABSTRACT

Objective: The indirect immunofluorescent assay (IFA) remains the gold standard for antinuclear antibody (ANA) testing. This test is accepted to be the most sensitive screening test for the detection of ANA. However, a drawback to the ANA test is the subjective nature of determining positive and negative results. The objective of this study was to determine if use of an automated digital imaging microscope improves agreement among multiple readers, and to assess improvement in the reproducibility of interpretation.

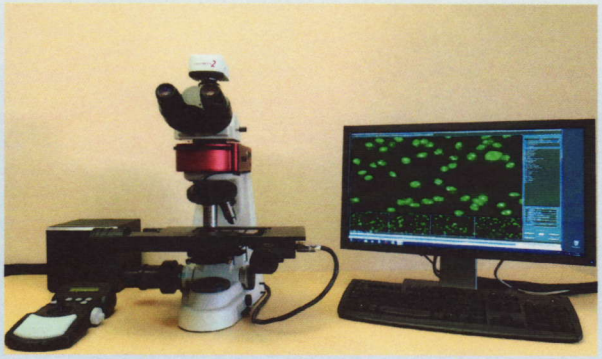
Methods: The instrument used in this study was the Immuno Concepts Image Navigator. Samples were human sera obtained from clinical laboratories in Europe and the United States. Sera were tested by IFA on HEp-2000 cells. Slides were read by experienced laboratory scientists using standard techniques, and were also screened using the Image Navigator system. Three independent observers assessed the images that were obtained on the Image Navigator. In the first phase of the study, the agreement among multiple readers was assessed, both by traditional manual techniques and the automated system. In the second phase, we compared reproducibility of interpretation by single readers.

Results: In phase one, the data show that multiple readers of the same slides agreed 93% of the time using traditional techniques, and they agreed in 97% of cases using the Image Navigator ($p<0.05$). In phase two, within-reader agreement was determined to be greater than 99% for each of three individuals using the Image Navigator.

Conclusion: The Image Navigator improves the reproducibility of the IFA screening test for ANA.

INSTRUMENT

The instrument used in this study was the Immuno Concepts Image Navigator. It is based on a conventional fluorescent microscope with a motorized stage, high-intensity LED light source, digital camera, computer, and proprietary software. The Image Navigator system identifies the slide, locates the HEp-2000 cells on the wells, focuses on the cells, and captures four fields within each well. These images are stored in the appropriate patient file on the computer. After all of the images are captured, the software sorts the samples into three groups, "controls", "possibly negative", and "possibly positive." The instrument first presents the control samples, so the user can quickly review and verify the quality control for that run. The next presentation is a grid view of the images from each of the samples that the system has classified as "possibly negative." The user reviews these images to verify that all are ANA negative. If an image appears to be positive, or if the reviewer wants to examine it more closely, it is transferred to the positive review pool with a single mouse click. After the user has determined that the remaining images all represent ANA negative samples, a single mouse click sends that result to the appropriate patient files. Finally, the "possibly positive' images are reviewed. In this review, four images of each sample are presented, the user examines them, determines the pattern(s) that are present, and enters this information by mouse clicks. At this time, additional tests and titers can be ordered on the sample. After all samples have been reviewed and verified, reports are printed, and the files are transferred to the Laboratory information System.



PATIENT SAMPLES

Samples were human sera obtained from clinical laboratories in the United States and Europe. All sera were submitted to the laboratories for routine ANA testing, but the laboratories were blinded as to the identities and diagnoses of the patients. A total of 148 sera were used in this study. All sera were tested using Immuno Concepts HEp-2000 transfected cells in an indirect immunofluorescent assay. Slides were read by three experienced laboratory scientists using standard techniques.

STUDY DESIGN

A search of the pertinent literature for the past three decades revealed that there has not been a published study of variability among readers of the same ANA samples, nor has there been a published study of reproducibility of ANA results within one reader from one time to another. In phase one of the study, we assessed the agreement among readers of ANA slides, using convention fluorescent microscopy and the automated Image Navigator system. We performed ANA screens on all 148 samples, and these slides were read by three individuals using a conventional fluorescent microscope. The samples were then randomized by an individual who was not involved in the reading of the slides, the ANA screens were repeated, and the same three readers assessed the slides. The samples were randomized a second time by the individual who was not involved in the reading of the slides, the ANA screens were repeated, and the same three readers assessed the slides for the third time. We assessed the agreement in determining positive and negative ANA results among multiple readers.

These same three sets of slides were processed on the Image Navigator, and the images were assessed by the same three scientists who had read the slides manually. Again, the agreement in determining positive and negative ANA results among multiple readers using the Image Navigator was assessed.

In phase two, we assessed within-reader agreement for both conventional reading and the automated system.

The data were collated by the individual who had randomized the samples and were analyzed for positive agreement, negative agreement, overall agreement, and Kappa statistics.

RESULTS

Table 1. Comparison of results for 3 independent observers reading indirect immunofluorescent Antinuclear Antibody tests using conventional methods.

Positive/Negative Assessment	Number of Samples
All 3 observers called negative	103
Two positive / one negative	7
One positive / two negative	3
All 3 observers called positive	35

Complete agreement among three observers: 93.2%, Kappa statistic: 0.54

Table 2. Comparison of results for 3 independent observers reading indirect immunofluorescent Antinuclear Antibody tests using the automated Image Navigator system.

Positive/Negative Assessment	Number of Samples
All 3 observers called negative	108
Two positive / one negative	2
One positive / two negative	2
All 3 observers called positive	36

Complete agreement among three observers: 97.3%, Kappa statistic: 0.78

Table 3. Within-reader reproducibility for three separate readings of indirect immunofluorescent Antinuclear Antibody tests using the automated Image Navigator system.

	Reader A	Reader B	Reader C
Positive/Negative Assessment	N	N	N
All 3 observations called negative	110	109	109
Two positive / one negative	0	1	1
One positive / two negative	1	0	1
All 3 observations called positive	37	38	37
Complete agreement among three observations	99.3%	99.3%	98.6%

CONCLUSION

The Image Navigator system provides an automated method for screening IIF ANA samples. The system improves agreement among readers, and reproducibility for individual readers.