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OPTIMIZATION OF THE ANTINUCLEAR ANTIBODY INDIRECT IMMUNOFLUORESCENCE TEST ON HEP-2 CELLS

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BACKGROUND

The indirect immunofluorescence assay on HEp-2 cells (HEp-2/IFA) is the most used method for screening antibodies to the nucleus and other cell compartments. A major caveat of HEp-2/IFA is the low specificity, as positive results also occur in some healthy individuals and patients with nonautoimmune chronic inflammatory diseases, apparently because HEp-2/IFA detects low-avidity and low-titer natural autoantibodies. We hypothesize that a modification of HEp-2/IFA with elution of low-avidity antibodies will increase the diagnostic specificity of the test.

MATERIAL AND METHODS

Six chaotropic solutions (NaCl-1M, 1.5M, 2M; Urea-2M, 3M, 4M) were tested to elute autoantibodies in the first washing step (after serum incubation) in the HEp-2/IFA procedure. Elution protocols with NaCl-2M and Urea-4M showed the best performance. Randomly selected samples from 200 healthy individuals (HI), 200 patients with nonautoimmune diseases (NAID) and 200 patients with diverse systemic autoimmune diseases (SARD) were processed in the standard HEp-2/IFA and in the modified NaCl-2M and Urea-4M assays, respectively. Reactivity was scored as nonreactive, weak/moderate (1+/2+) and strong (3+/4+).

RESULTS

In the HI group, 42 (21%) samples were reactive in the standard protocol, 27 (13.5%) and 12 (6%) of which became nonreactive with the NaCl-2M protocol and the Urea-4M protocol, respectively, resulting in 64% ($p < 0.001$) and 28% ($p < 0.02$) reduction in reactivity, respectively. In the NAID group, 88 (44%) samples were positive in the standard protocol, 37 (18.5%) and 22 (11%) of which became nonreactive with the NaCl-2M protocol and the Urea-4M protocol, respectively, resulting in 42% ($p < 0.05$) and 25% ($p < 0.05$) reduction in reactivity, respectively. In the SARD group, 190 (95%) samples were reactive in the standard protocol, 40 (20%) and 7 (3.5%) of which became nonreactive with the NaCl-2M protocol and the Urea-4M protocol, respectively, resulting in 21% ($p < 0.05$) and 3% ($p = 0.2$) reduction in reactivity, respectively. In the three groups, most of the samples that became nonreactive in the elution protocols had a nuclear fine speckled pattern and weak/moderate reactivity in the conventional protocol. Lastly, considering the three groups altogether, the sensitivity/specificity for SARD diagnosis was 95% and 67.5% with the standard protocol, 75% and 83.5% with the NaCl-2M protocol and 91.5% and 76% with the Urea-4M protocol (Table 1).

Table 1. Diagnostic performance of standard and modified HEp-2 IFA.

HEp-2/IFA	Reactive samples		Sensitivity	Specificity	PPV [*]	NPV ^{**}
	SARD (n = 200)	xxxxx				
Standard	190	130	95.0%	67.5%	59.4%	96.4%
NaCl-2M	150	66	75.0%	83.5%	69.4%	87.0%
Urea-4M	183	96	91.5%	76.0%	65.6%	94.7%

*PPV: positive predictive value; **NPV: negative predictive value.

CONCLUSION

These results indicate that the elution protocols with NaCl-2M and Urea-4M improved the diagnostic performance of the HEp-2/IFA test, by promoting higher specificity and positive predictive value. However, as expected, such improvement occurs at the expense of a decrease in diagnostic sensitivity and negative predictive value. The elution protocol with NaCl-2M performed better than the elution protocol with Urea-4M.

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