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KEY WORD : AUTOIMMUNE DISEASE / IMMUNOBLOTTING / ANTINUCLEAR ANTIBODY DETECTION

WATCHARAKORN WANGNAI: PREPARATION OF ANTIGEN STRIPS FOR THE DETECTION OF ANTINUCLEAR ANTIBODIES (ANAs). THESIS ADVISOR: SUPHANEE SARNTIVJAI M.Sc., VIRAPONG PRACHAYASITTIKUL Ph.D. 106 p. ISBN 974-662-100-9

The purpose of this study was to prepare ANA/A-DNA dot blot antigen strips for ANAs and A-DNA screening test and ANA western blot strip for the detection of ANA specific to ENA. The antigen sources were HEp-2 cell extract and RTE which were found to be the appropriate antigens among the four antigen sources studied. Deoxyribonucleic acid for immunodot blot A-DNA detection was kDNA extracted from *C. luciliae*. The immunoenzyme staining of ANA/A-DNA dot blot strips were evaluated as compared to FANA -HEp 2 cell and IFA using *C. luciliae* in the detection of ANAs and A-DNA. The sensitivity and specificity of ANA detection by HEp-2 cell ANA dot blot strips were 92.24% and 90.11% of those of FANA and when using RTE dot blot strips the sensitivity and specificity were 92.31% and 85.34% of FANA's respectively. The sensitivity and specificity of A-DNA detection by using kDNA dot blot was 100% and 96.00% as compared to using *C. luciliae* IFA. Therefore, this ANA/A-DNA dot blot antigen strip could be used for the ANA and A-DNA screening test in laboratories where fluorescent microscopes are not available.

When 116 ANA positive and 68 ANA negative sera were examined for specific ANAs by Ouchterlony double immunodiffusion using RTE as the source of ENA and anti-Sm, anti-RNP, anti-SSA, anti-SSB anti-Jo-1 and anti Scl 70 as the prototype sera, anti RNP was found in 3 MCTD sera and anti-Sm/RNP was found in 1 SLE sera. HEp-2 cell Western blot strip was used for the detection of specific ANAs by immunoperoxidase staining. Anti Sm/RNP (28/68 kDa) was found in 17 SLE; anti RNP (68 kDa) was found in 7 SLE, 19 MCTD and 1 scleroderma sera; anti Scl 70 (70 kDa) was found in 2 SLE and 5 scleroderma sera; anti SSA/anti SSB (48/60 kDa) was found in 1 Sjogren's syndrome serum; anti Jo-1 was found in 1 PM/DM serum. Uncorrelated bands to the six reference sera used were found from 23 SLE, 4 MCTD, 5 scleroderma, 21 RA, 10 ITP/AIHA and some ANA negative sera. When RTE Western blot strip was used, the same results as shown by HEp-2 cell Western blot strips were produced except for anti-Scl70 which was found in only 3 scleroderma sera. Uncorrelated bands to the reference sera used were found from 25 SLE, 4 MCTD, 7 scleroderma, 21 RA, 10 ITP/AI HA and also some ANA negative sera.

ANAs Western blot strip using HEp-2 cell extract provided higher frequency in the detection of anti Scl 70 than using RTE while the efficacy of detecting other ANAs elicited the same results. The immunoenzyme staining ANAs Western blot strips gave more positives in the detection of ANAs and was more economical to perform as compared to the Ouchterlony method. This study suggested that the ANAs Western blot strips could replace the currently used immunodiffusion method.