Utility of the HEp-2000 antinuclear antibody substrate

We write with regards to the recently published consensus paper on antinuclear antibody (ANA) staining patterns by Damoiseaux *et al* which was most informative.[1] We were surprised, however, that there was no mention or discussion of the HEp-2000 substrate (Immunoconcepts, Sacremento, California, USA) despite the acknowledgement that anti-SS-A/Ro60 is frequently missed on indirect immunofluorescence assay (IIFA).[1] The HEp-2000 substrate is a modified HEp-2 substrate that is transfected with Ro60 cDNA and hence, over-expresses this antigen.[2] It produces a bright nuclear/nucleolar staining pattern under IIFA when anti-SS-A/Ro60 antibodies are present (figure 1), and is one of the most commonly detected extractable nuclear antigen antibodies.[3]

The HEp-2000 substrate is a sensitive way to detect anti-SS-A/Ro60 antibodies that may be otherwise missed on conventional immunoassays, such as immunoblotting and the standard HEp-2 substrate.[4 5] However, it is not a perfect screen as the HEp-2000 substrate may still miss detection of anti-SS-A/Ro60 sera[6 7] indicating that at least a second method for anti-SS-A/Ro60 and other antibody detection is still required.[8] For other ANA specificities, HEp-2000 performs similarly to the standard HEp-2 substrate with no significant impact on the detection of other ANA fluorescent patterns[9 10] and in fact, improved sensitivities.[5]

It is important to maximise the chances of identifying anti-SS-A/Ro60, for the antibody, as an example, has significant implications for fertile women for the development of neonatal lupus erythematosus which may have devastating sequelae.[11] We therefore recommend others to consider the HEp-2000 substrate if employing the IIFA method for ANA detection.

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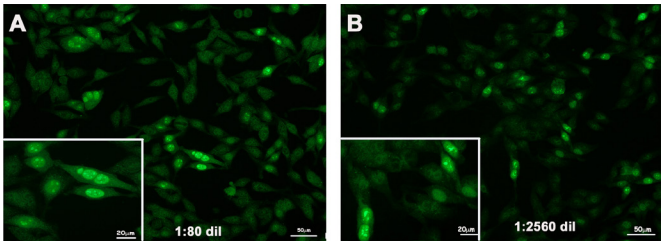

Figure 1 Typical anti-SSA/Ro60 nuclear/nucleolar staining on the HEp-2000 substrate using serum specific for anti-Ro60 at (A) 1:80 dilution and (B) 1:2560 dilution. Even with a 32-fold dilution, the staining is still quite distinct at 1:2560 (B). Insets represent enlarged versions of representative areas on the micrograph.

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REFERENCES

1 Damoiseaux J, Andrade LEC, Carballo OG, *et al*. Clinical relevance of HEp-2 indirect immunofluorescent patterns: the International consensus on ANA patterns (ICAP) perspective. *Ann Rheum Dis* 2019;78:879–89.
2 Keech CL, McCluskey J, Gordon TP. Transfection and overexpression of the human 60-kDa Ro/SS-A autoantigen in HEp-2 cells. *Clinical Immunology and Immunopathology* 1994;73:146–51.
3 Wang K-Y, Yang Y-H, Chuang Y-H, *et al*. The initial manifestations and final diagnosis of patients with high and low titers of antinuclear antibodies after 6 months of follow-up. *Journal of Microbiology, Immunology and Infection* 2011;44:222–8.
4 Fritzler MJ, Miller BJ. Detection of autoantibodies to ss-a/ro by indirect immunofluorescence using a transfected and overexpressed human 60 kd Ro autoantigen in HEp-2 cells. *J. Clin. Lab. Anal.* 1995;9:218–24.
5 Kidd K, Cusi K, Mueller R, *et al*. Detection and identification of significant Anas in previously determined ANA negative samples. *Clin Lab* 2005;51:517–21.
6 Bossuyt X, Frans J, Hendrickx A, *et al*. Detection of anti-SSA antibodies by indirect immunofluorescence. *Clin Chem* 2004;50:2361–9.
7 Choi MY, Clarke AE, St.Pierre Y, *et al*. Antinuclear antibody-negative systemic lupus erythematosus in an international inception cohort. *Arthritis Care Res*. 2018.
8 Hoffman IEA, Peene I, Veys EM, *et al*. Sensitivity of ANA indirect immunofluorescence testing for the detection of anti-ENA antibodies. *Arthritis Res* 2002;4(Suppl 1).
9 Keech CL, Howarth S, Coates T, *et al*. Rapid and sensitive detection of anti-Ro (SS-A) antibodies by indirect immunofluorescence of 60kDa Ro HEp-2 transfectants. *Pathology* 1996;28:54–7.
10 Peene I, Van Ael W, Vandenbossche M, *et al*. Sensitivity of the HEp-2000 substrate for the detection of Anti-SSA/Ro60 antibodies. *Clinical Rheumatology* 2000;19:291–5.
11 Lee LA. Cutaneous lupus in infancy and childhood. *Lupus* 2010;19:1112–7.