

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, Sacramento, 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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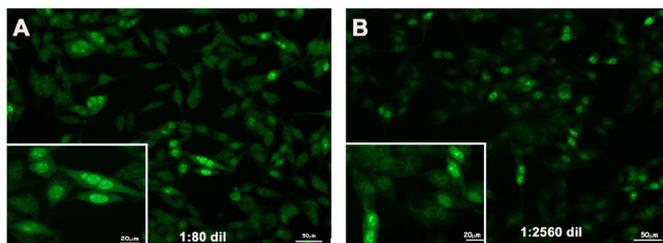


Figure 1 Typical anti-SSA/Ro60 nuclear/nucleolar staining on the HEp-2000 substrate using serum specific for anti-Ro60 at (A) 1:80 dilution and (B) 1:2560 dilution. Even with a 32-fold dilution, the staining is still quite distinct at 1:2560 (B). Insets represent enlarged versions of representative areas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

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