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## Comment on: Clinical utility of ANA measured by ELISA compared with ANA measured by immunofluorescence

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**Matters arising:** Comment on the letter by GA Maguire *et al.*: Clinical utility of ANA measured by ELISA compared with ANA measured by immunofluorescence. *Rheumatology* 2009;48:1013-1014

Sir,

We read with interest the recently published study by Maguire and co-workers on the clinical utility of antinuclear antibody testing (ANA) in a real-world rheumatology setting (1). By comparing indirect immunofluorescence (IF) microscopy with an enzyme-linked immunosorbent assay (ELISA), they reached the conclusion that ELISA is the preferable method. This subject is a matter of debate since many years (2,3). The opinion of Maguire and co-workers is based in their finding that sera from 169 patients (14%), out of 1239 analysed during a 12-month period, tested IF-positive but ELISA-negative. This result did not relate to self-reported symptoms suspect of systemic lupus erythematosus (SLE) or any other systemic inflammatory disease compared to age- and sex-matched controls testing ANA-negative with both IF and ELISA. However, we have a few objections to the study design. First and foremost, regarding ANA as a criterion for SLE, the 1982 ACR classification states that “an abnormal titer of antinuclear antibody by immunofluorescence or an equivalent technique” is required (4). As shown in many studies, IF and ELISA are not equivalent regarding ANA results. Thus, at present only IF-ANA qualifies as an ACR classification criterion, but solely when it exceeds the cut-off limit for an *abnormal antibody titre*, *i.e.* in practice at a serum level above the 95<sup>th</sup> percentile in a healthy blood donor material. Maguire and co-workers screened for IF-ANA at a serum dilution of 1:40. Assuming their IF test was performed with up-to-date equipment, screening for ANA at this high serum concentration will undoubtedly result in a huge number of positive reactions below abnormal titre (5,6). On the other hand, with an appropriate cut-off level for IF-ANA, the analysis is likely to result in a point prevalence of ANA far below 95% among patients with established SLE (7). A caveat regarding the IF-ANA test used by Maguire and co-workers is that the secondary antibody in their diagnostic kit was achieved using whole human IgG molecules rather than IgG-Fc fragments. Thus, the fluorochrome-labelled detection antibody may recognize not only IgG, but also light chains of other immunoglobulin isotypes, potentially capturing also IgM-class ANA, which is more common than IgG-ANA among healthy blood donors (8). Based upon >3000 serum samples sent for routine ANA testing, although not scrutinizing the clinical rationale to perform the test, we came to the conclusion that IgG-class specific IF-ANA must remain the gold standard for ANA screening (using proper cut-off limits), at least as long as the present ACR classification criteria are in use (5). In their paper, Maguire and co-workers do not state the proportion of ELISA-positive sera testing IF-ANA negative. In our hands, this is a bigger problem than the reverse. Nevertheless, in addition to IF microscopy using HEp-2 or HEp-2000 cells as nuclear antigen substrates, we advocate the use of supplementary antigen-specific assays (*e.g.* ELISA) in order not to miss out patients positive for anti-SSA/Ro60 antibodies!

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The authors declare no conflicts of interest.

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