Response to: "Antinuclear autoantibodies: discordance among four different assays" by Pacheco *et al*

We thank Dr Pacheco and colleagues for their comments¹ on our article² on the variation of testing for antinuclear antibodies (ANA) by indirect immunofluorescence (IIF) in the context of systemic lupus erythematosus (SLE). Along with findings described in other letters,^{3–8} the observations by Pacheco and colleagues on three different line immunoassays (LIA) and an ELISA indicate that, as in the case of other technologies, LIAs can show considerable discordance among different kits that are currently available. This study also shows that results by LIA can differ from those obtained by an ELISA.

Differences among ANA tests can have a variety of causes including assay conditions as well as the origin and biochemical properties of test antigens. Thus, for proteins, antigens may be a preparation purified from tissue, a cloned product or a peptide sequence. Perhaps, not surprisingly, testing with these different antigen preparations may produce divergent results. While the full explanation for differences among assay formats and tests is uncertain, positing that an assay approach (ie, the IIF) is the gold standard may not be fully supported by the data.

In view of our results and those of others, we believe that ANA testing needs to be re-evaluated in terms of emerging technologies as well as the reasons for which testing is now performed. Importantly, ANA positivity is now viewed as a necessary criterion for the classification of a patient with SLE; it is also increasingly used as a criterion for entry into clinical trials for patients with 'active, autoantibody positive' SLE. For these purposes, test variation can have a profound impact. The implications for serological testing for clinical research are also great since efforts to associate specific ANAs with clinical manifestations (eg, anti-Ro and neonatal lupus) or immunological features (eg, antibodies to RNA binding proteins and the interferon signature) will be impacted by testing variation.

We believe that greater standardisation and harmonisation of testing are critical and may perhaps require new study designs for assay validation to accord better with current uses for testing. For example, the presence of ANA positivity at the time of lupus diagnosis may differ from that during established disease when changes in ANA expression may occur because of effects of treatment as well as evolution of the immunological profile over time. As such, test validation may need to include samples from patients with varying disease duration, severity and treatment history, preferably associated with well-annotated clinical data. It is also important to consider differences in 'real world' testing with that in the research setting. In the research setting, experts or otherwise highly experienced laboratory personnel may conduct the testing by IIF. Such individuals may be more adept at recognising different staining patterns or low positive responses than individuals working in clinical or commercial laboratories.

ANA testing has moved from an important focus of fundamental research into the world of routine clinical testing with

sometimes an inadequate appreciation of the vagaries of these assays and the type of variation that can occur. We think that the responses to our paper that have been published in the journal have been important in revealing problems in current serological testing and the urgent need to find real world solutions.

David S Pisetsky,¹ Diane M Spencer,¹ Peter E Lipsky,² Brad Rovin³

¹Department of Medicine and Immunology, Duke University Medical Center and Medical Research Service, VA Medical Center, Durham, North Carolina, USA

²RILITE Research Institute, Charlottesville, Virginia, USA

³Division of Nephrology, The Ohio State University, Wexner Medical Center, Columbus, Ohio, USA

Correspondence to Dr David S Pisetsky, Department of Medicine and Immunology, Duke University Medical Center, Durham, NC 27705, USA; david.pisetsky@duke.edu

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