primarily on qualitative agreement. Although seemingly very straightforward, these types of comparisons are more difficult than they appear, largely because estimated sensitivities and specificities and the agreement between methods is heavily dependent on the cutoffs used to differentiate between positive and negative.

Historically, IIF has been considered the most sensitive method for identifying patients with AARDs. In a 2009 position statement on ANA testing methods, the American College of Rheumatology identified IIF as the “gold standard for ANA testing” primarily based on its high sensitivity (>95%) for the diagnosis of SLE (5). However, the statement also acknowledges that the specificity of ANA by IIF is a limitation. In a cohort of patients for whom ANA testing was ordered as part of routine clinical care, we demonstrated that IIF at a titer cutoff of 1:40 had a sensitivity of 94% for the general diagnosis of AARDs (6). This was higher than the sensitivity of either EIA or MIA, at 74% and 67%, respectively. However, the IIF’s higher sensitivity was at the expense of specificity, which, at the 1:40 cutoff, was only 43%. In comparison, the corresponding EIA and MIA specificities were 80% and 87%, respectively. When we increased the cutoff for IIF to 1:80, the specificity improved to 62% but the sensitivity decreased to 84%.

Some data suggest that the titer of the ANA may help in distinguishing between patients with and without AARDs. In a study from 2011, Mariz et al. demonstrated that 45.8% of positive ANAs in healthy controls had a titer of 1:80, while 88.5% of ANA-positive AARD patients had an ANA titer ≥1:320 (7). Many laboratories that perform ANA by IIF are moving away from screening at the 1:40 dilution, opting for improved specificity even with some loss in sensitivity. When labs use higher screening dilutions, the sensitivities of IFIs are on par with those of EIAs and MIAs. Although IFIs have the capability of maximizing sensitivity, from a practical perspective, EIAs and MIAs provide a good balance of sensitivity and specificity.

IIF’s sensitivity is attributed to its broad antigen specificity. This method detects antibodies against any of the hundreds of nuclear and cytoplasmic antigens present in a cell. However, not all antigen specificities are relevant for the diagnosis of AARDs. For example, the DFS pattern appears almost exclusively in patients with no evidence of an AARD (7). It has been suggested that the presence of the DFS pattern could be used to rule out an AARD in an individual with a positive ANA. The antigen specificity associated with this pattern has been identified as lens epithelial-derived growth factor, also referred to as DFS70 (8). Further studies have confirmed that monospecificity for DFS70 in the context of a DFS pattern is not consistent with an AARD. This pattern, and perhaps others like it that have yet to be characterized, may help to address some of the specificity challenges associated with ANA testing by IIF.

PERFORMANCE CONSIDERATIONS FOR ANA METHODOLOGIES

When labs are considering which ANA method to implement, availability of a qualified technologist to perform the testing is likely a significant concern. Other key considerations include throughput, workflow, and automation of a method.

Although automation of immunological testing has not reached the level of chemistry platforms, significant strides have been made over the last decade, particularly with EIAs and MIAs. EIAs can be performed manually, although more often than not, labs perform this testing on semi-automated or automated platforms. The semi-automated platforms may dilute patient samples and add reagents to the plate, but a technologist’s intervention might be required to wash and move the plate to an absorbance reader. A fully automated system processes an EIA in its entirety, only requiring technologists to load samples and reagents. Most MIA systems are also fully automated.

In addition, MIAs have the advantage of being random access, which facilitates improved workflows. In contrast, EIAs are batched, which, for labs with lower volumes of ANA orders, could have a negative impact on workflow and on turnaround times. Another advantage of MIA systems is they offer labs the opportunity to expand their test menus. Most MIA systems are not limited to ANA testing, and have reagents available for other autoimmune conditions (celiac disease, antiphospholipid syndrome, and vasculitis) and for infectious diseases (Epstein-Barr virus, HIV, and herpes simplex virus). Being able to perform additional testing and maximize an instrument’s utilization could make an MIA system an attractive option.

Historically, IIF has been the ANA method requiring the most clinical technologist resources and expertise,