Variation in antinuclear antibody detection by automated indirect immunofluorescence analysis

Pisetsky *et al* reported on assay variation in the detection of antinuclear antibodies (ANA) in sera of patients with established systemic lupus erythematosus (SLE).¹ The authors determined ANA in sera from 103 patients with established SLE using three different validated and widely used immunofluorescence assays (IFA) (from ImmunoConcepts, Inova Diagnostics and Bio-Rad), an ELISA and a bead-based multiplex assay (both from Bio-Rad). With IFA, the frequency of ANA negativity varied from 4.9% to 22.3%. Part of the samples (11.7% and 13.6%) were negative with ELISA and multiplex assay, respectively. This study showed differences among IFA ANA kits which might have implications for eligibility for clinical trials.¹

In a correspondence to the paper of Pisetsky *et al*,¹ Dr Mahler discussed some relevant points related to ANA detection,² including the substantial interobserver variability inherent to IFA. Accordingly, Dr Mahler recommended to use automated interpretation systems to reduce variability and subjectivity.²

We evaluated variation in ANA detection by automated IFA systems. Three samples (sample 1: homogeneous 1:320; sample 2: fine speckled 1:80; sample 3: negative) were distributed to 31 Belgian laboratories that use automated IFA systems and 27 laboratories participated (NOVA View n=12 (Inova Diagnostics, San Diego, USA); EUROPattern n=6 (Euroimmun, Lübeck, Germany); G-Sight n=7 (Menarini, Firenze, Italy); Image Navigator n=2 (ImmunoConcepts, Sacramento, California, USA)). Each sample was determined at least four times in different runs (the majority was determined at least eight times) according to the instructions of the manufacturer. The fluorescence intensity units (reported as light intensity units (LIU) or probability index) (reported as light intensity units (LIU) or probability index)

Figure 1 Fluorescence intensity (light intensity unit (LIU) or probability index (PI)) of antinuclear antibodies (ANA) by automated immunofluorescence assays (IFA). Three samples (sample 1: homogeneous 1:320; sample 2: fine speckled 1:80; sample 3: negative) were analysed by NOVA View (n=12: L1–L12), Image Navigator (n=2: L13–L14) or G-Sight (n=7: L15–L21). All laboratories used a screening dilution of 1:80 except for L17 that used a screening dilution of 1:40. All samples were diluted by a pipetting robot (QUANTA-Lyser, PhD, Sprinter, Zenit UP, Beeline, Helmed). For G-Sight, HEP-2000 or HEP-2 was used as substrate (as indicated). The cut-off for positivity was 49 LIU for NOVA View, 49 LIU for Image Navigator, <8 PI (negative) and >50 PI (positive) for G-Sight (L15–L17, L19–L21) or 20 PI for G-Sight (L18). All samples were run at least four times in different runs (the majority was determined at least eight times) according to the instructions of the manufacturer. The fluorescence intensity units (reported as LIU or PI) are shown. Statistical analysis of differences in fluorescence intensities between laboratories was evaluated by analysis of variance with Bonferroni correction for multiple comparisons. For NOVA View, there were significant differences between L1 versus L4 (p=0.044), L8 (p=0.001), L9 (p=0.002); L2 versus L8 (p=0.024) and L7 versus L8 (p=0.009), L9 (p=0.022) for sample 1, between L2 versus L4 (p=0.003), L8 (p=0.039), L9 (p=0.022); L7 versus L3 (p=0.034), L4 (p=0.026), L5 (p=0.033), L8 (p=0.003), L9 (p=0.018) and L11 versus L2 (p=0.022), L7 (p=0.007), L10 (p=0.005) for sample 2 and between L1 versus L4 (p=0.005), L8 (p=0.001), L11 (p=0.018) for sample 3. For G-Sight, there were differences between L15 versus L17 (p=0.001) and L16 versus L17 (p<0.001) for sample 1 and between L16 versus L17 (p=0.002) and L19 versus L21 (p=0.013) for sample 3. NOVA View estimates a pattern-specific end point titre based on the 1:80 screening dilution, a feature called single well titre. The single well titre varied between 1:160 and 1:640 for sample 1, between <1:80 and 1:320 for sample 2 and between <1:80 and 1:160 for sample 3.

Figure 2 Intralaboratory performance of antinuclear antibodies (ANA) by automated immunofluorescence assays (IFA) for NOVA View. Light intensity units (LIU) values for an analysis of sample 1 in 2016 and in 2017 (the time difference between the two determination ranged between 7 and 19 months). The results shown are from at least five (2016) or seven (2017) determinations in different runs. For each laboratory (L), the first set of results shown were obtained in 2016 and the second set of results in 2017.

are shown in figure 1, except for EUROPattern that does not report fluorescence intensity. The results not only show variation in the way fluorescence intensity is reported by different companies but also (1) variation in between-run imprecision between automated IFA systems from the same manufacturer and (2) variation in fluorescence intensity results between instruments from the same manufacturer. For instance, for NOVA View, the coefficient of variation for sample 1 varied between 8.6% and 39.7% and there were statistically significant differences (after correction for multiple comparisons) in LIU values between laboratories (figure 1 and its legend). Also for G-Sight, significant differences between laboratories were found. For sample 2, six laboratories using NOVA View and three laboratories using G-Sight found the sample to be negative in 10%–80% of the between-run determinations for NOVA View and in 12.5%–62.5% of the between run determinations for G-Sight. The other laboratories found the sample to be positive for all determinations, with differences in fluorescence intensities between laboratories. For sample 3 (negative sample), three laboratories using NOVA View and four laboratories using G-Sight found positive results in 10%–62.5% of the determinations for NOVA View and in 10%–100% of the determinations for G-Sight. Some of these results were due to artefacts as expert visual review scored 60%–80% of the false positive determinations for NOVA View negative.

For a selection of patterns, NOVA View allows to estimate the end titre from the analysis of the 1:80 dilution (see legend of figure 1). EUROPattern (performed by six laboratories) does not provide a measure of fluorescence intensities. It scored sample 1 positive for all determinations ($n=61$) (with the estimated end titre varying between 1:80 and 1:1280) and sample 2 for 59% of the determinations ($n=51$) (with the estimated end titre varying between <1:80 and 1:160). Sample 3 was negative in 92% of the determinations ($n=60$).

Pattern assignment by automated IFA was correct in 76%, 95% and 100% of the determinations by NOVA View, EUROPattern and G-Sight for sample 1. For sample 2, the values were, respectively 32%, 100% and 100%. This indicates that pattern

assignment by automated systems should be verified by an experienced technician or immunologist.

Sample 1 had been used to evaluate interinstrument variability for NOVA View in a previous study³ performed 1 year before the current study. This gave us the possibility to evaluate constancy of the results over a period of 7–19 months for 11 of the 12 NOVA View users. The results are represented in figure 2 and show statistically significant differences between LIU values obtained at two different time points for 6/11 laboratories. Of note, for five laboratories, no statistical significant differences between the two determinations were observed, which illustrates the potential of automated IFA analysis to provide reproducible results over a longer time period.

Taken together, although we could demonstrate reproducible ANA results for some laboratories, our results indicate variation in ANA detection by automated IFA systems. We not only found variation between automated IFA analysis using instruments from different manufacturers but also between instruments from the same manufacturer. Efforts should be undertaken to harmonise automated IFA analysis. This could include the use of standards, calibration of the instruments and monitoring of the quality of the slides and reagents.

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