

Measuring anti-dsDNA Antibodies

Anti-dsDNA antibodies are an important diagnostic marker in a number of autoimmune conditions, including systemic lupus erythematosus (SLE). The presence of high levels of these autoantibodies is associated with worse outcomes, including incidence of severe complications such as nephropathy. Declining levels of anti-dsDNA antibodies are also used to monitor therapeutic efficacy. Unfortunately, anti-dsDNA antibodies are well known to be challenging analytes for clinical assays, and no consensus has yet emerged on the best method for measuring them. Here we directly compare a number of different methods for measuring anti-dsDNA antibodies in clinical sera.

Comparing Assays

Comparing different assays which measure (or claim to measure) the same thing is more challenging than it first appears. First, a series of clinical samples need to be analysed using the different assay methodologies. Next, the results are compared with each other and (ideally) with a gold-standard.

Selecting a gold-standard assay for anti-dsDNA antibodies is difficult – each lab has their own ‘favourite’, reflecting the lack of consistency even among the well-established methodologies. Most experts, however, cite either the **Farr assay** or the **Crithidia assay** as the nearest there is to a gold-standard.

Because these gold-standard assays are resource intensive (and technically demanding to perform reproducibly), many labs have sought an alternative. A number



Using Crithidia (inset) to detect anti-dsDNA antibodies by immunofluorescence

of suppliers now offer assays that utilise ELISA methodology. Better still, Pronostics have combined measurement of anti-dsDNA antibodies with eight other assays for anti-nuclear antibodies using our Ultraplex™ technology to combine nine different assays into one well, minimising reagents and labour, reducing costs still further.

We have assembled a panel of 152 sera with a wide range anti-dsDNA antibody levels (but with a higher incidence of dsDNA positivity than an unselected cohort), and compared 5 different assays: the Farr assay and Crithidia assay are compared with **Ultraplex™ ANAscreen** from Pronostics, as well as the two market-leading ELISA assays (designated ELISA A and ELISA B).

Just how similar?

It is not uncommon to see the use of correlation coefficients in comparisons of assays methods. This approach, however, can be unhelpful. Correlation analysis is designed to test whether there is a linear association between variables or whether they are entirely unrelated, with the correlation coefficient a measure of the extent of the relationship among variables. Of course, it is exceptionally unlikely that two assays purported to measure the same thing are entirely unrelated, so the magnitude and significance of the correlation coefficient are insensitive measures of the degree of agreement between the assays – crucially not helping the user decide if the two assays are *sufficiently similar*. Moreover, the correlation coefficient is related to the range of the samples measured.

What is the alternative? Increasingly, scientists are using **Bland-Altman** plots to describe the similarity between two alternative measures of the same factor. These plots give 95% confidence intervals for the outcome of a second assay applied to the same sample. For example, if assay A yields an answer of 100 units, then assay B might give an answer for that sample between 90 and 110 units 95% of the time (if assay B is very similar to assay A), or alternatively it might give answers ranging from 50 to 150 units (if the two were less similar).

For these reasons, Pronostics use Bland-Altman statistics to compare different assays, giving the user considerably more information about the properties of our assays than the use of correlation coefficients.

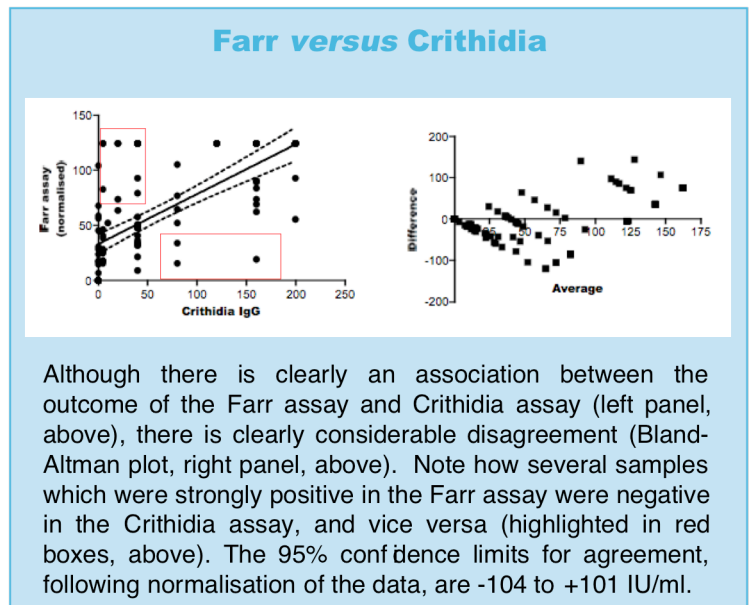
Want to know more about Bland-Altman statistics for assay comparisons? Contact Pronostics today.

“Not-so-gold” Standards

First, we compared the results obtained using the Farr and Crithidia assays (box, right). It is immediately clear why there is no universal agreement about which is the gold-standard assay. The extent of the agreement between them, estimated from the Bland-Altman plots, is not particularly impressive. Some 10/152 samples were classified as positive for dsDNA antibodies by one of the two gold-standard assays and negative with the other. It is entirely unclear which of these two results should be considered correct without performing large-scale clinical trials.

Five Equivalent Assays

Next, we compared the results from the two market-leading ELISA assays and the Ultraplex™ ANAscreen assay with the Farr and Crithidia assays. The data presented below use the Farr assay as the gold-standard comparator (because it yields a continuous, quantitative measure in contrast to the Crithidia immunofluorescence assay), but very similar results are obtained adopting the Crithidia assay as the gold standard. The Limits of Agreement, calculated from the Bland-Altman plots (not shown) for all of the assays, are very similar (see box below). This tells us that ANAscreen, as well as the ELISA assays, give results which are as similar to the Farr assay as does the Crithidia assay.

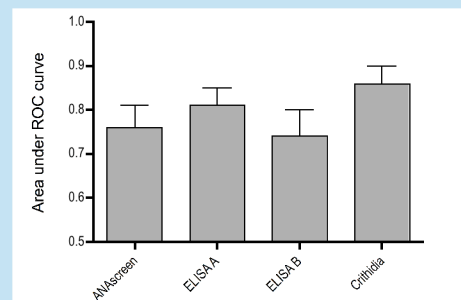


ANAscreen, ELISAs and Crithidia versus Farr

	ANAscreen	ELISA A	ELISA B	Crithidia
Mean difference (95% CI)	-1 (-8 to 7)	-8 (-14 to -2)	-28 (-35 to -20)	n/a
Lower limit of agreement (95% CI)	-84 (-97 to -71)	-78 (-90 to -67)	-111 (-124 to -98)	-104 (-122 to -87)
Upper limit of agreement (95% CI)	82 (70 to 96)	63 (52 to 74)	56 (43 to 69)	101 (87 to 122)

The Bland-Altman limits of agreement for ANAscreen, two market-leading ELISA assays and the Crithidia assay each predicting the outcome of the Farr assay are shown in the Table above. ELISA B has a tendency to over-read, but otherwise the tests all perform similarly well.

An alternative way to compare the performance of the assays is to use the area under the Receiver Operating Characteristic (ROC) curve, shown here (right). Once again, it is clear that all the assays perform similarly.



Another way of comparing assays is to use the area under the Receiver-Operator Curve (ROC). Here, assays that always give exactly the same answer would have a value of 1.0, while two unrelated assays would have a value of 0.5. ANAscreen, like the two market-leading ELISAs, predicts the result from a Farr assay to a similar extent as does the Crithidia assay. Since all the assays compared here are broadly equivalent, the decision as to which assay to adopt depends on issues such as a cost and ease of use. It is important to remember, though, that all the assays for dsDNA are imperfect (the area under the ROC curves are less than 0.9 in every case) and that some samples will inevitably be found for which the five assays tested here disagree profoundly.

All the assays we tested for measuring anti-dsDNA antibodies are broadly equivalent, but major discrepancies between any of them are still possible (reflecting the well-known difficulties with assays for anti-dsDNA antibodies).