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Workshop reports

The second International Workshop on the Standardisation of Insulin Autoantibody (IAA) Measurement

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The first International Workshop on Insulin Autoantibodies (IAA) in Perth, 1987, found marked variation in the measurement of individual sera between laboratories, which in part could be ascribed to differences between liquid and solid phase assays. The workshop concluded that differences might be minimised by (a) correcting for non-displaceable signal, and (b) interpolating from a standard curve.

The second workshop therefore asked participants to provide raw data in duplicate (counts per min or optical density units) before and after incubation with an excess of human insulin and to run a standard curve. This workshop addressed the need for competition/displacement data, the value of a standard curve, and a proposal to use non-dimensional units, based on assay precision, with which to compare IAA results.

Twenty-seven laboratories participated. Standard and reference sera were supplied together with 37 coded sera from nine different sources. Samples incorporated (1) 11 normal sera; (2) 13 thought to be IAA positive by the contributing laboratories; (3) 5 thought to be insulin antibody (IA) positive from insulin treated diabetic patients; (4) a reference serum (IA) undisclosed in four dilutions, and (5) the standard serum (IA) undisclosed in three dilutions. Complete data on which analysis of displacement could be performed was available from 14 laboratories: eight from laboratories using radiobinding assay (RBA) and six using enzyme-linked immunosorbant assay (ELISA). Data for analysis of signals interpolated through a standard curve was provided by 18 laboratories (nine RBA, nine ELISA).

Analysis of results before displacement with cold insulin showed substantial and variable non-specific binding of all sera, including normals in many assays, particularly ELISA. The removal of non-specific binding reduced the intensity and scatter of signals of the normal sera substantially. Thus, only data from laboratories performing assays with displacement, and thereby providing specific signals, were used for further analysis.

In addition to interpolating signals through a standard curve to obtain arbitrary IAA units, a separate analysis was performed based on assay precision. This precision unit, the standard deviation score (SDS), was derived from the specific test signal - mean of normal signals: standard deviation of normal signals. SDS is not as much a measure of signal intensity as of the degree of certainty with which results can be distinguished from normal, and therefore deemed to be positive.

The frequency distribution of the SDS for the 11 normal sera is shown in Figure 1. The figure was constructed by establishing the mean and standard deviation of specific signals from the normal sera in each laboratory, returning to assign each result a standard deviation score accordingly, and calculating the frequency with which each SDS occurred in the laboratories overall. RBA and ELISA behaved similarly and the distribution of the signals, except for a single serum from one laboratory, lay within ±5.5 SDS. Samples were considered positive if their signal exceeded +5.5 SDS.

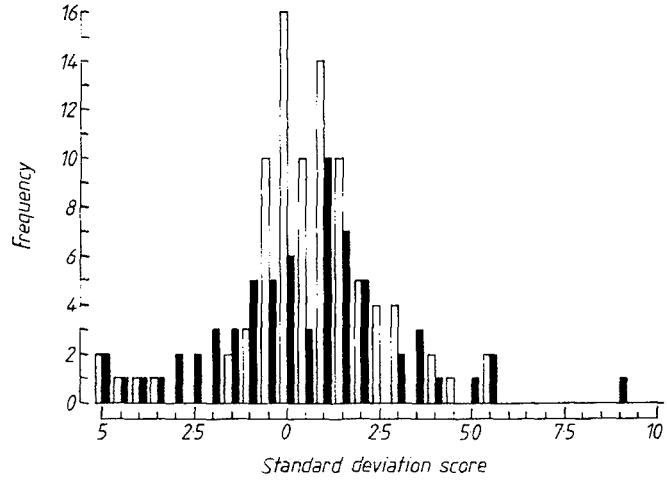

Fig. 1. The frequency distribution of insulin binding signals obtained for normal sera (n = 11) by laboratories using radiobinding assay (RBA) (□) and ELISA (■) expressed as standard deviations. Fourteen laboratories are represented

The mean interpolated IAA binding unit and SDS for each of the undisclosed standard (IA serum) and reference (IAA serum) dilutions are shown in Figures 2a and 2b alongside data from normal sera, sera deemed to be IAA positive and sera deemed to be IA positive by their contributing laboratories. Figure 2b shows clear differences between RBA and ELISA when measuring the standard and reference sera. The differences in assay response to the standard serum inevitably resulted in bias of the interpolated IAA units expressed in Figure 2a. The use of SDS avoids methodological bias; each column in Figure 2b represents simply the mean precision with which the laboratories could distinguish the relevant sera from normal.

Four strong sera, No.'s 7, 14 and 18 (IAA) and No. 8 (IA) are not included in the figure because they required dilution and could not be given an SDS without corresponding dilution of the normal sera. Two of the sera (No.'s 7 and 14) were human insulin specific IAA from patients with the insulin autoimmune syndrome. Using the criterion of 5.5 SD, all laboratories deemed serum 7 positive, 13 of the

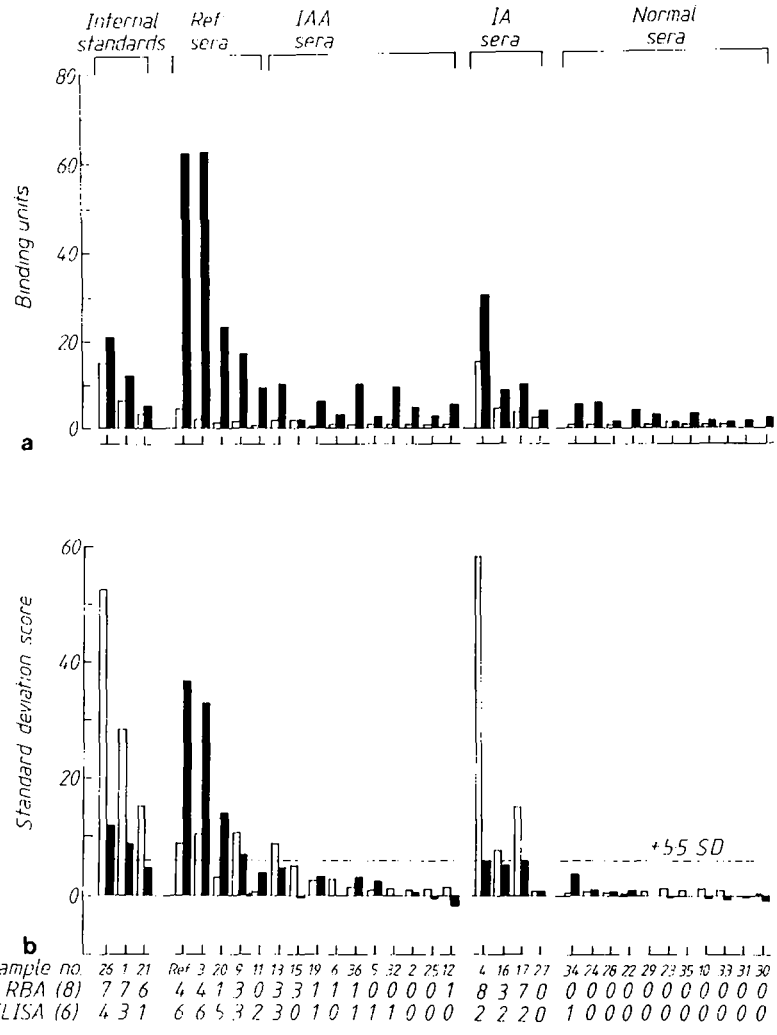

Fig. 2. **a** The mean signal intensity in IAA binding units, corrected for non-specific binding, obtained from laboratories using RBA (□) and ELISA (■) for representative sera from a diabetic (internal standards), relatives of Type 1 (insulin-dependent) diabetic patients (IAA sera), diabetic patients (IA) and normal subjects. Sera No.'s 3, 20, 9 and 11 were undisclosed serial dilutions of the reference (IAA) serum designated "Ref", and sera No.'s 26, 1 and 21 were undisclosed dilutions of the standard serum. Fourteen laboratories are represented. **b** The same data as in 2a, expressed as mean standard deviation score. The frequency with which laboratories (RBA or ELISA) designated each serum positive according to a cut-off of 5.5 SD is given below the graph

14 deemed No.'s 8 and 14 positive and 8/8 RBA but 2/6 ELISA laboratories deemed serum No. 18 positive (data based on SDS scores and subject to the limitations of dilution noted above).

In addition to systematic differences between RBA and ELISA for some sera, there was marked variation in the interpretation of positive or negative according to the criteria in Figure 1 within each assay group. This was particularly true for sera with weak IAA. As yet, it is unclear whether these differences in interpretation were due to differences in analytical sensitivity between assays or to differences in antibody binding characteristics. The future use of SDS should resolve this issue.

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